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(54) Title: CREATION OF DIVERSITY IN POLYPEPTIDES

(57) Abstract: The inventors realized that the diversity generated by conventional methods may be limited by steric hindrance between amino acid residues in the three-dimensional structures of the resulting polypeptides. The steric hindrance may occur between amino acid residues at widely different positions in the amino acid sequences, e.g. between residues in two different domains of the 3D structure, and resulting polypeptides which include such steric hindrance may never be observed in the conventional recombination methods because they may be expressed in poor yields or may have poor activity or stability. The inventors developed a method to identify and alleviate such steric hindrance in the resulting polypeptides. In an alignment of the three-dimensional structures, steric hindrance is indicated when residues from two different structures are located within a certain distance. Pairs of residues at corresponding positions in the amino acid sequences are not considered, and residues close to the surface (high solvent accessibility) are considered to be less prone to steric hindrance.

CREATION OF DIVERSITY IN POLYPEPTIDES

FIELD OF THE INVENTION

The present invention relates to a method of constructing a hybrid polypeptide from two or more parent polypeptides in order to create diversity. It also relates to hybrid polypeptides constructed by this method.

BACKGROUND OF THE INVENTION

The prior art describes methods of creating diversity by recombination of DNA sequences encoding two or more polypeptides, followed by transformation of a suitable host organism with the recombined DNA sequence and screening of the transformants for enzymatic activity. The recombination may be random or directed. WO 1995022625; US 6368805; J.E. Ness et al., Nature Biotechnology, vol. 20, Dec. 2002, pp. 1251-1255; M.C. Saraf et al., 4142-4147, PNAS, March 23, 2004, vol. 101, No. 12.

SUMMARY OF THE INVENTION

The inventors realized that the diversity generated by conventional methods may be limited by steric hindrance between amino acid residues in the three-dimensional structures of the resulting polypeptides. The steric hindrance (also referred to as "structural stop codon") may occur between amino acid residues at widely different positions in the amino acid sequences, e.g. between residues in two different domains of the 3D structure, and resulting polypeptides which include such steric hindrance may never be observed in the conventional recombination methods because they may be expressed in poor yields or may have poor activity or stability.

The removal of "structural stop codons" can result in improved expression and/or stability of the protein of interest, or in ultimate case expression at all of protein of interest. For example in combining of two or more proteins, i.e. combining multiple hybrids of two or more proteins using various DNA techniques e.g. using shuffling techniques as known in the art (WO9522625, WO9827230 and WO2000482862) the removal of "structural stop codons" from one or more of the included proteins will improve the expression and/or stability of the proteins, and/or create access to a novel diversity not found by other shuffling or hybrid techniques. Combination of protein sequences will often result in accommodation of different sized residues and homologous positions, but not always. Sometimes clashes will occur and especially in the core of the protein. The removal of "structural stop codons" results in novel diversity due to allowance of new region combinations not seen because of presence of "structural stop codons", which otherwise may result in a non functional or non expressed protein.

The inventors developed a method to identify and alleviate such steric hindrance in the resulting polypeptides. In an alignment of the three-dimensional structures, steric hindrance is indicated when residues from two different structures are located within a certain distance. Pairs of residues at corresponding positions in the amino acid sequences are not taken into consideration since only one of the two residues is expected to be present in the recombined polypeptide. Pairs of residues are not taken into consideration if one or both is glycine or if one or both side chains is close to the surface (indicated by a high solvent accessibility) as the residue may be able to reposition to avoid the potential clash.

Accordingly, the invention provides a method of constructing a polypeptide, compris-10 ing:

- a) selecting at least two parent polypeptides each having an amino acid sequence and a three-dimensional structure,
- b) structurally aligning the three-dimensional structures, thereby aligning amino acid residues from different sequences,
- c) selecting a first amino acid residue from one structure and a second residue from another structure, such that:
 - i) the two residues are not aligned in the superimposition,
 - ii) a non-hydrogen atom of the first residue and a non-hydrogen atom of the second residue are located less than 2.7 Å apart, and
 - iii) each of the two residues is not Glycine and has a side chain having less than 30 % solvent accessibility, and
 - d) substituting or deleting the first and/or the second residue such that the substitution is with a smaller residue, and
 - e) recombining the amino acid sequences after the substitution, and
- 25 f) preparing a DNA-sequence encoding the polypeptide of step e) and expressing the polypeptide in a transformed host organism.

Further the invention relates to a polypeptide which has at least 80%, 85%, 90%, 95% or 98% or 99% identity to SEQ ID NO: 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25. The invention also relates to a polynucleotide encoding any of the polypeptides.

BRIEF DESCRIPTION OF DRAWINGS

- Fig. 1 shows an alignment of various known CGTase sequences. Details are given below.
- Fig. 2 shows the results of a comparison of two 3D structures. The upper sequence is 1qho for the maltogenic alpha-amylase Novamyl (SEQ ID NO: 17), and for the lower sequence

is 1a47 for a CGTase (SEQ ID NO: 5). Details are described in Examples 1 and 2.

Fig. 3 and 4 shows hypothetical sequences with "structural stop codons". Details are described in Examples 6 and 7.

DETAILED DESCRIPTION OF THE INVENTION

5 Parent polypeptides

According to the invention, two or more parent polypeptides are selected, each having an amino acid sequence and a three-dimensional structure. The parent polypeptides may in particular be selected so as to be structurally similar, e.g. each pair having a amino acid identity of at least 50 %, e.g. at least 60 %, 70 % or 80 %. Amino acid identity may be determined as described in <u>US 6162628</u>.

In another preferred embodiment the structurally similar parent polypeptides have a homology of at least 50 %, e.g. at least 60 %, 70 %, 80 %, 90% or 95%. Homology may be determined as described in WO 2004067737, i.e. by using the GAP routine of the UWGCG package version 9.1.

The parent polypeptides may be polypeptides having biological activity, structural polypeptides, transport proteins, enzymes, antibodies, carbohydrate binding modules, serum albumin (e.g. human and bovine), insulin, ACTH, glucagon, somatostatin, somatotropin, thymosin, parathyroid hormone, pituitary hormones, somatomedin, erythropoietin, luteinizing hormone, interleukin, chorionic gonadotropin, hypothalamic releasing factors, antidiuretic hormones, thyroid stimulating hormone, relaxin, interferon, thrombopoeitin (TPO) and prolactin.

The enzyme may have an active site, e.g. a catalytic triad, which may consist of Ser, Asp and His. The parent enzymes may be selected so as to have identical residues in the active site.

Three-dimensional structure

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Three-dimensional structure is meant to be a known crystal structure or a model structure.

The 3D structure of each polypeptide may already be known, or it may be modeled using the known 3D structures of one or more polypeptides with a high sequence homology, using an appropriate modeling program such as Homology, Modeller or Nest. The 3D model may be optimized using molecular dynamics simulation as available, e.g., in Charmm or NAMD. The optimization may particularly be done in a water environment, e.g. a box or sphere.

The Homology, Modeller and Charmm software is available from Accelrys Inc., 9685 Scranton Road, San Diego, CA 92121-3752, USA, http://www.accelrys.com/. The Nest soft-

distributed free of charge at http://trantor.bioc.columbia.edu/programs/jackal/index.html. The NAMD software is available at http://www.ks.uiuc.edu/Research/namd/.

Structural alignment of 3D models

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The 3D models may be structurally aligned by methods known in the art. The structural alignment may be done by use of known software. In the structurally aligned models, pairs of residues from different sequences are considered to be aligned when they are located close to each other. The following software may be used:

DALI software, available at http://www.ebi.ac.uk/dali/

CE software available at http://cl.sdsc.edu/ 10

STAMP software available at

http://www.compbio.dundee.ac.uk/Software/Stamp/stamp.html

Protein 3Dhome at http://www-lecb.ncifcrf.gov/~tsai/

Yale Gernstein Lab - spare parts at http://bioinfo.mbb.yale.edu/align/

Structural alignment server at http://www.molmovdb.org/align/ 15

In the case of enzymes having an active site, the structural alignment may be a superimposition of the structures based on the deviations of heavy atoms (i.e. non-hydrogen atoms) in the active sites, e.g. by minimizing the sum of squares of deviations. Alternatively, the superimposition may be done so as to keep deviations between corresponding atoms below 0.8 Å, 20 e.g. below 0.6 Å, below 0.4 Å,, below 0.3 Å or below 0.2 Å.

Selection of amino acid residues

Steric hindrance ("potential clashes") between two amino acid residues is indicated if a heavy atoms (i.e. non-hydrogen) of the two residues are located less than 2.7Å, 2.5 Å or 2.0 Å apart, particularly less than 1.7 Å, 1.5 Å, 1.2 Å, 1.1 Å or 1.0 Å apart, with the following excep-25 tions:

Two residues aligned with each other in the structural alignment (pairs of residues at corresponding positions in the amino acid sequences) are not taken into consideration since only one of the two residues is expected to be present in the recombined polypeptide.

Pairs of residues are not taken into consideration if one or both is glycine.

Pairs of non-glycine residues are not taken into consideration if one or both side chains has more than 20 %, 25 % or 30 % solvent accessibility as a high solvent accessibility is taken as an indication that the residue may be able to reposition to avoid the potential clash. Solvent accessibility can be calculated by use of the DSSP program, available from Centre for Molecular and Biomolecular Informatics, University of Nijmegen, Toernooiveld 1, P.O. Box 35 9010, 6500 GL Nijmegen, +31 (0)24-3653391, http://www.cmbi.kun.nl/gv/dssp/. The DSSP

program is disclosed in W. Kabsch and C. Sander, BIOPOLYMERS 22 (1983) pp. 2577-2637. The residue total surface areas of the 20 natural amino acids are tabulated in Thomas E. Creighton, PROTEINS; Structure and Molecular Principles, W.H. Freeman and Company, NY, ISBN: 0-7167-1566-X (1984).

To confirm the severity of the potential clash, a local alignment of the two 3D structures may then be made by aligning all residues within a distance of 10 Å.

The steric hindrance may be identified by a comparison of two complete sequences in order, particularly severe clashes (less than 1.2, 1.1 or 1.0 Å apart), to identify potential clashes that may arise no matter how the two sequences are recombined.

Alternatively, the comparison may be made between two partial sequences to be combined in a hybrid, and in this case a larger limit may be used for the distance (less than 2.7 Å, 2.5 Å, 2.0 Å, 1.7 Å or 1.5 Å).

Amino acid substitution

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When a potential clash between two residues has been identified, one or both resi-15 dues is substituted with a smaller residue. In this connection, the residues are ranked as follows from smallest to largest: (an equal sign indicates residues with sizes that are practically indistinguishable):

The substitution may be such that the two residues after the substitution can form a hydrogen bond, a salt bridge or a cysteine bridge.

Recombination of amino acid sequences

After making amino acid substitutions to alleviate potential clashes, the substituted amino acid sequences are recombined. The recombination may be done by designing hybrids or by gene shuffling.

Hybrids may be constructed by switching from one sequence to another between aligned residues. Once constructed, the hybrids can be produced by conventional methods by preparing a DNA sequence encoding it and expressing it in a transformed host organism.

Alternatively, genes can be prepared encoding each substituted amino acid sequence, by shuffling the genes by known methods, transforming a suitable host organism with the shuffled genes. The shuffling can be done, , e.g., as described in <u>WO 1995022625</u>.

In the case of the parent polypeptides being enzymes, the transformants can be screened for enzymatic activity.

Enzymes

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The parent enzymes may have hydrolase, oxidoreductase or transferase activities, e.g. activities such as protease, lipolytic enzyme, glycosyl hydrolase, laccase, oxidoreductases with oxygen as acceptor (e.g. glucose oxidase, hexose oxidase or galactose oxidase), glycosyl transferase, esterase, cellulase, xylanase, amylase, isoamylase, pullulanase, branching enzyme, pectate hydrolase, cyclodextrin glucanotransferase, or maltogenic alpha-amylase activity. One or more of the parent enzymes may have a carbohydrate-binding domain.

The method may particularly be applied to two or more structurally similar enzymes, e.g. belonging to the same family in a structural classification of enzymes. Thus, they may belong to the same structural family for glycosyl hydrolases and glycosyl transferases as described, e.g., in the following literature. The enzymes may be of family 13 and may particularly include a maltogenic alpha-amylase and a cyclodextrin glucanotransferase.

- Henrissat B., A classification of glycosyl hydrolases based on amino-acid sequence similarities. Biochem. J. 280:309-316 (1991).
- Henrissat B., Bairoch A. New families in the classification of glycosyl hydrolases based on amino-acid sequence similarities. Biochem. J. 293:781-788 (1993).
- Henrissat B., Bairoch A. Updating the sequence-based classification of glycosyl hydrolases. Biochem. J. 316:695-696 (1996).
- Davies G., Henrissat B. Structures and mechanisms of glycosyl hydrolases. Structure 3:853-859 (1995).

The parent enzymes may be lipolytic enzymes belonging to the same homologous family as described at http://www.led.uni-stuttgart.de/families.html. The 3D structures of the lipolytic enzymes may all include a so-called "lid" in open or closed form.

The enzymes may be proteases or peptidases belonging to the same family or sub25 family as described by MEROPS in "the Peptidase Database", available at
http://merops.sanger.ac.uk/. The proteases may be subtilases, e.g. belonging to the same subgroup as described by Siezen RJ and Leunissen JAM, 1997, Protein Science, 6, 501-523; one
of these sub-groups is the Subtilisin family.

CGTase

The cyclodextrin glucanotransferase (CGTase) may have an amino acid sequence as shown in SEQ ID NOS: 1-16 and may have a three-dimensional structure found under the following identifier in the Protein Data Bank (www.rcsb.org): B. circulans (1CDG), alkalophilic Bacillus (1PAM), B. stearothermophilus (1CYG) or Thermoanaerobacterium thermosulfurigenes (1CIU, 1A47). 3D structures for other CGTases may be constructed as described in Example 1 of WO 9623874.

Fig. 1 shows an alignment of the following known CGTase sequences, each identified by accession number in the GeneSeqP database and by source organism. Some sequences include a propertide, but only the mature peptide is relevant for this invention.

SEQ ID NO: 1. aab71493.gcg B. agaradherens

SEQ ID NO: 2. aau76326.gcg Bacillus agaradhaerans

SEQ ID NO: 3. cdg1_paema.gcg Paenibacillus macerans (Bacillus macerans).

SEQ ID NO: 4. cdg2_paema.gcg Paenibacillus macerans (Bacillus macerans).

SEQ ID NO: 6. aaw06772.gcg *Thermoanaerobacter thermosulphurigenes* sp. ATCC 53627 (SEQ ID NO: 3)

SEQ ID NO: 7. cdgt_bacci.gcg Bacillus circulans

SEQ ID NO: 8. cdgt_bacli.gcg Bacillus sp. (strain 38-2)

SEQ ID NO: 9. cdgt_bacs0.gcg Bacillus sp. (strain 1011)

SEQ ID NO: 10. cdgt_bacs3.gcg Bacillus sp. (strain 38-2)

SEQ ID NO: 11 cdgu_bacci.gcg Bacillus circulans

SEQ ID NO: 12. cdgt_bacsp.gcg *Bacillus sp.* (strain 17-1, <u>WO 2003068976</u>) (SEQ ID NO: 4)

SEQ ID NO: 13. cdgt_bacoh.gcg Bacillus ohbensis

SEQ ID NO: 14. cdgt_bacs2.gcg Bacillus sp. (strain 1-1)

SEQ ID NO: 15. cdgt bacst.gcg Bacillus stearothermophilus

SEQ ID NO: 16. cdgt_klepn.gcg Klebsiella pneumoniae

To develop variants of a CGTase without a known 3D structure, the sequence may be aligned with a CGTase having a known 3D structure. An alignment for a number of CGTase sequences is shown in Fig. 2. Other sequences may be aligned by conventional methods, e.g. by use the software GAP from UWGCG Version 8.

Maltogenic alpha-amylase

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The maltogenic alpha-amylase (EC 3.2.1.133) may have the amino acid sequence shown in SEQ ID NO: 17 (in the following referred to as Novamyl), having the 3D structure described in <u>US 6162628</u> and found in the Protein Data Bank with the identifier 1QHO. Alternatively, the maltogenic alpha-amylase may be a Novamyl variant described in <u>US 6162628</u>. A 3D structure of such a variant may be developed from the Novamyl structure by known methods, e.g. as described in T.L. Blundell et al., Nature, vol. 326, p. 347 ff (26 March 1987); J. Greer, Proteins: Structure, Function and Genetics, 7:317-334 (1990); or Example 1 of <u>WO 9623874</u>.

Use of hybrid polypeptide

The hybrids may be useful for the same purpose as the parent enzymes.

Thus, a hybrid of a maltogenic alpha-amylase and a cyclodextrin glucanotransferase may form linear oligosaccharides as an initial product by starch hydrolysis and a reduced amount of cyclodextrin and may be useful for anti-staling in baked products.

A hybrid of laccases and/or other enzymes belonging to EC 1.10.3 may be useful for e.g. hair dyeing or reduction of malodor.

EXAMPLES

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Example 1: Comparison of complete sequences

10 Superimposition of parent enzymes

Two glycosyl hydrolases of family 13 were selected. One was a maltogenic amylase (Novamyl) having the amino acid sequence shown in SEQ ID NO: 17 and having a 3D structure published under number 1 QHO. The other was a CGTase having the amino acid sequence shown in SEQ ID NO: 5 and the 3D structure 1A47, and this was also taken to represent the structure of the highly homologous CGTase having the sequence SEQ ID NO: 6. The two 3D structures were superimposed so as to align the active sites, and the alignment of residues of the two sequences is shown in Fig. 2 Aligned residues shown vertically above each other, with gaps inserted to separate non-aligned residues.

Identification of potential clashes

The two structures were analyzed, and the following unaligned residues were identified as having a side chain with less than 30 % solvent accessibility and with a heavy atom less than 1.5 Å (or less than 1.0 Å) apart from a heavy atom of a residue in the other structure. The following pairs of residues were found to come within 1.0 Å. The potential clashes are shown as CGTase residue and atom, Novamyl residue and atom, and distance in Å:

D209	OD2	A676	CB	0.89
L261	CD1	K270	NZ	0.93
D267	CG	N266	0	0.94
D267	OD1	N266	0	0.48
M307	CE	L286	CD1	0.77
H503	CD2	K7	NZ	0.97
T509	OG1	Y574	CZ	0.65
V626	СВ	Y181	CZ	0.41
V626	CG1	Y181	ОН	0.99

V626	CG2	Y181	CD2	0.76
K651	NZ	P592	CG	0.35

The above residues are marked by asterisks in Fig. 2.

Example 2: Comparison of complementary sequences

To design hypothetical hybrids, residues in a partial sequence of Novamyl (SEQ ID NO: 17) were compared with residues in the complementary part of the CGTase sequence (SEQ ID NO: 6), and residues with heavy atoms located less than 1.7 Å apart were identified. The potential clashes are shown as in Example 1. The identified residues are marked with asterisks in Fig. 2.

	Novamyl 1-494	1 + CG	Tase 495-683	<u>3</u>		
	H5	03	CD2	K7	NZ	0.97
			0	Y317	ОН	1.68
	V6	26	СВ	Y181	CZ	0.41
	CGTase 1-494	+ Nov	amyl 495-686	<u>5</u>		
	D	3	С	R545	NH2	1.36
			OD2	A676	СВ	0.89
10	Novamyl 1-499	9 + CG	Tase 500-683	<u>3</u>		
	LIF	503	CD2	K7	NZ	0.97
				Y317	OH	1.68
		26	СВ	Y181	CZ	0.41
	VC	20	OD	1101	02	· · · ·
	CGTase 1-499	9 + Nov	<u>/amyl 500-686</u>	<u> </u>		
)3	С	R545	NH2	1.36
	D2	209	OD2	A676	СВ	0.89
	Novamyl 1-41	0 + CG	Tase 410-68	<u>3</u>		
	H	503	CD2	K7	NZ	0.97
		575	0	Y317	ОН	1.68
		626	СВ	Y181	CZ	0.41
	Novamyl 1-37	8 + CG	Tase 378-68	<u>3</u>		
	N	409	OE1	R354	N	1.63
		503	CD2	K7	NZ	0.97
	N:	575	0	Y317	ОН	1.68

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V626	СВ	Y181	CZ	0.41					
Novamyl residues	Novamyl residues 1-204 + CGTase residues 207-683								
W219	CZ2	L75	CD2	1.66					
H503	CD2	K7	NZ	0.97					
V626	СВ	Y181	CZ	0.41					
CGTase residues 1-139 and 207-683 + Novamyl residues 131-204									
V626	СВ	Y181	CZ	0.41					

Example 3: Construction of hybrids

Hybrids were constructed with the following combinations of Novamyl residues and 5 CGTase residues (SEQ ID NO: 6) and with substitutions of Novamyl residues as indicated to alleviate potential clashes. For comparison, similar variants were constructed without substitutions.

· Residues	Novamyl substitu- tions
Novamyl 1-494 + CGTase 495-683	K7S +Y181A
CGTase 1-494 + Novamyl 495-686	R545S
Novamyl 1-499 + CGTase 500-683	K7S +Y181A
CGTase 1-499 + Novamyl 500-686	R545S
Novamyl 1-410 + CGTase 410-683	K7S +Y181A
Novamyl 1-378 + CGTase 378-683	K7S +Y181A
Novamyl 1-204 + CGTase 207-683	K7S, W107F
CGTase 1-139 + Novamyl 131-204 + CGTase 207-683	Y181A
Novamyl 1-204 + CGTase 207-683	K7S, W107F, Y181A
Novamyl 1-204 + CGTase 207-683	K7S, Y181A

The first eight of the above hybrids are found in SEQ ID NO: 18 to SEQ ID NO: 25.

10 Example 4: Screening of hybrids for amylase activity

Four hybrids of the previous example were produced by preparing a DNA-sequence encoding the hybrid and expressing the hybrid in a transformed organism cultivating a transformant, and the amylase activity was assayed by letting the culture broth act on Phadebas (dye-labelled substrate, available from Pharmacia) and measuring the absorbance at 650 nm.

The amylase assay was made at pH 5.5 at two different temperatures: 50°C and 60°C. Reference hybrids without substitutions were included for comparison.

Decidure	Novamyl substitu-	ABS (650 nm)	ABS (650 nm)	
Residues	tions	pH 5.5, 60°C	pH 5.5, 50°C	
Novamyl 1-410 + CGTase 410-683	_	0.01	0.01	
Novamyl 1-410 + CGTase 410-683	K7S, Y181A	0.49	1.66	
Novamyl 1-378 + CGTase 378-683	-	0.01	0.01	
Novamyl 1-378 + CGTase 378-683	K7S, Y181A	0.16	0.37	
CGTase 1-139 + Novamyl 131-204 + CGTase 207-683	_	0.06	0.02	
CGTase 1-139 + Novamyl 131-204 + CGTase 207-683	Y181A	0.21	0.07	

Example 5: Baking with hybrids.

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Further two hybrids were produced by cultivating a transformant and tested for baking. The two hybrids are:

BaHy1: CGTase (SEQ ID NO: 6) residue 1-139 + Novamyl (SEQ ID NO: 17) residue 131-204 + CGTase (SEQ ID NO: 6) residue 207-683; and

BaHy2: Novamyl (SEQ ID NO: 17) residue 1-577 + CGTase (SEQ ID NO: 6) residue 580-683 + Y181A mutation in Novamyl.

The effect of the two hybrids in straight dough was compared to that of CGTase with respect to a number of parameters: Softness of breadcrumb, elasticity, and mobility of free water.

Approximately 1 mg/kg of flour was dosed.

The two hybrids improve the softness of breadcrumb as compared to CGTase.

15 The two hybrids improve the elasticity as compared to CGTase.

BaHy2 improves the mobility of free water as compared to CGTase, whereas BaHy1 has the same effect as CGTase.

Example 6: Structural stop codons – impact on diversity.

This example illustrates the possible outcome of a hybridization between two proteins 20 having the sequences SeqA and SeqB (figure 3):

If combination sites (marked with |) comprises a "structural stop codon" (marked with X), the resulting protein not be expressed properly or maybe even not at all. Segment 14 in SeqA and segment 7 in SeqB indicates such potential clashes due to the presence of "structural stop codons". The result will be a lowering of the diversity, as combinations containing these two

segments most likely not will be able to accommodate the clashes and therefore not be present in the diversity of protein molecules.

If X in SeqA and /or SeqB is made smaller the accommodation might result in a functional protein. Accommodation may also be obtained by changing the shape or charge of the residue e.g. I to L and D to N. The "structural stop codon" can also be removed by inserting the proper match of residues by mutating the particular residues and/ or mutating the surrounding residues around the clashing residues thus creating accommodation. Smaller residues can be found in the list; G < A=S=C < V=T < P < L=I=N=D=M < E=Q < K < H < R < F < Y < W.

If the "structural stop codon" gives 100% non-functional protein - the lowering of diversity is 25% for one "structural stop codon" residue pair - compared to the situation without any "structural stop codons". That is the diversity for the segments are $2^{20} = 1048576$ and for the clashes it is $2^{18} = 262144$.

Example 7: Structural stop codons – impact on diversity when combining more than two proteins.

In this example we have three proteins illustrated by SEQ1, SEQ2 and SEQ3 (figure 4). SEQ1 has a "structural stop codon" with SEQ2 called X. SEQ1 has a "structural stop codon" with SEQ3 called Y. The diversity will hereby be lowered dramatically as exemplified above. We will have the common equation for the number of non-"structural stop codon" containing proteins termed D for diversity in the cases where the "structural stop codons" pairs are found in separate segments not containing other "structural stop codons" and the number of segments are higher or equal to the number of pairs:

Equation I: $D = N^{K} - P^{*}N^{(K-2)}$

where D is diversity without "structural stop codons", N the number of proteins, K the number segments, and P the number of pairs (ie. X, Y and Z).

For other situations e.g. with "structural stop codons" in the same segment or other situations other equations can be derived.

Using equation I we get D to be 2/3 for the numbers shown in present example and for the numbers in shown in the above example we get 0.75. Consequently the diversity may be increased significantly by removing "structural stop codons".

Example 8: Structural stop codons – impact on extending combination possibilities for proteins with low homology to a better result.

One important aspect is the possibility of combining more distant related proteins by hybridisation or shuffling techniques and not only closely related proteins. The combination by hybridisation or shuffling techniques may go below the 90, or the 80, or the 70, or the 60, or the 50 percent homology level. At the upper level of homology, around 70-90 percent homology, the amount of diversity – meaning the number of active clones coming out of a hybridisation or shuffling experiment – or at the lower level around 50-80 percent homology creation of active clones at all might be the outcome.

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Example 9: Example on finding "structural stop codons" for combining proteins e.g. shuffling or hybrid formation.

The set of parent sequences are analyzed using the 3D structures. The 3D structures can be based on existing known structures or obtained by X-ray crystallography, NMR meth15 ods or modeled using appropriate modeling programs like NEST, MODELLER or HOMOLOGY. The two structures are superimposed by optimizing the RMSD of the C-alpha atom distances using a appropriate program as listed in the description. The superimposed structures
are analyzed for possible clashes between residues. For each type of atoms (a,b), where atom
a is in structure A and atom b is in structure B the distance d(a,b) between the atoms is calculated as the standard Euclidian distance. All atom pairs with distance smaller than a given predefined threshold are potentially structural clashes. A set of rules is imposed to filter out atom
pairs with distance smaller than the threshold which are not to be considered as clashes. The
rules are:

- i. Atom pairs that form part of the residue that are aligned in the alignment based on the superimposition are filtered out.
- ii. Atom pairs that form part of residues that are adjacent to aligned residue are filtered out.
- iii. Atom pairs where both atoms are backbone atoms are filtered out.
- iv Atom pairs that form part of residues that are both surface exposed are filtered out.

 Surface exposed can be computed based on the "solvent exposed surface area" computed by the DSSP-program by division by the standard accessibilities in the following list; A=62, C=92, D=69, E=156, F=123, G=50, H=130, I=84, K=174, L=97, M=103, N=85, P=67, Q=127, R=211, S=64, T=80, V=81, W=126 and Y=104. The threshold fro interatomic distances can be 3Å, or 2.7Å, or 2.5Å or 2.3Å, or 2.1Å or 2Å. The minimal relative surface exposed area for filtering out an atom pair is 20% or preferably 30% for each residue. The found clashes are visualized and inspected in a graphic display program.

Example 10: "Structural stop codons" for combining Protease – Subtilisin S8A

After the superimposition of the two X-ray structures of BPN' (1SBT – also disclosing the amino acid sequence) and Savinase (1SVN – also disclosing the amino acid sequence) using a suitable display software like INSIGHT II from Accellrys inc. a "structural stop codon" can be found i.e. a clash between to residues with distance lower than a certain threshold here 2.5Å. The residues giving a clash can be seen are located in the core of the two proteins and having the following residues below 2.5Å apart to I198 from Savinase structure 1SVN and I268 BPN' 3D structure 1SBT. Mutation of either 1SVN to I198V or A or G or T, or the SBT sequence to I268V or A or G or T will remove the interaction.

So for example making the hybrid construction 1SVN sequence A1-G219 and 1SBT sequence N218-Q275 should include the mutations suggested above to obtain the best result regarding expression.

Example 11: "Structural stop codons" for combining protease TY145 and Savinase

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After the superimposition of the two X-ray structures of TY145 (see patent application WO2004067737 A3, also disclosing the amino acid sequence (SEQ ID NO: 1)) and Savinase (1SVN – also disclosing the amino acid sequence) using a suitable display software like IN-SIGHT II from Accellrys inc. a "structural stop codon" can be found i.e. a clash between to residues with distance lower than a certain threshold here 2.1Å:

TY145 P308 clashes with Savinase I198

TY145 W101 clashes with Savinase M119

TY145 103 clashes with Savinase W113

Savinase Y263 clashes with TY145 Mainchain

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Example 12: "Structural stop codons" for combining lipases:

Two hybrid enzymes consisting of the N-terminal from Thermomyces lanuginosus lipase (TLL, SEQ ID NO: 26) and the C-terminal from Fusarium sp. lipase (KVL, SEQ ID NO: 27) have been constructed (Construct 1 and Construct 2). The point of crossover resides within conserved regions within the two enzymes. A study of the three-dimensional structure of Thermomyces lanuginosus lipase 1GT6 and a model of the Fusarium sp. lipase build based on the 1GT6 structure reveals two places of residue clashes when making the two hybrid constructs.

In general the following "structural stop codons" can be found:

35 TLL F142 clashes with KVL F136

TLL T64 clashes with KVL F24

TLL I222 clashes with KVL Y226

TLL F80 clashes with KVL I60

TLL F55 clashes with KVL A62

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The structural problem has been alleviated by introduction of the following mutations T64G and T64G/I222L into the two hybrid enzymes Construct 1 and Construct 2, respectively.

The constructs for two specific hybrids are (the numbers are taken for KVL and TLL protein sequences):

Construct 1. KVL 1-28 and TLL 29-269

Construct 2. KVL 1-28 and TLL 29-227 and KVL 225-267

Construct 3. KVL 1-28 and TLL 29-269 and TLL T64G

Construct 4. KVL 1-28 and TLL 29-227 and KVL 225-267 and TLL T64G and TLL I22L

The 3D structures of the KVL lipase was build using the Accelrys software HOMOLOGY program – other suitable software like NEST could also be used.

Example 13: "Structural stop codons" for combining laccases:

Analyzing the three dimensional structure of the Coprinus cinerius laccase (CLL, SEQ ID NO:28) and the three dimensional structure model of Myceliophthora thermophila laccase (MTL, SEQ ID NO: 29) build using the NEST software based on the Melanocarpus albomyces laccase structure (1GWO – also disclosing the amino acid sequence), it can be found that several "structural stop codons" can be found. Focusing on the core "structural stop codons" the following residues can found to be important to mutate. There are the following important "structural stop codons" that has to be removed before attempting shuffling of the two laccases of CCL and MTL:

MTL M301A and/or CCL F124L

CCL E239A or D

30 CCL E453A

MTL W464L

MTL W420F

There are besides the mentioned changes other important issues concerning the cystin bridges MTL C301/C267 and CCL C135/C222. Securing of no overlaps in theses regions are of great importance. To avoid the problems the following are a plausible way to go further::

MTL C379S/C345S and CCL C135G/C222V

Alternatively "transfer" CCL cystinbridge to MTL: MTL G193C/V281C.

Example 14: "Structural stop codons" for combining xylanases:

Analysing the three dimensional structure of the Bacillus agaradherens xylanase (BAX), having the X-ray structure 1QH7 (also disclosing the amino acid sequence), and the three dimensional structure of Bacillus halodurans xylanase (BHX) having the X-ray structure 1XNB (also disclosing the amino acid sequence), it can be found that several "structural stop codons" can be found. Focusing on the core "structural stop codons" the following residues can found to be important to mutate:

10 BAX R49 clashes with BHX Y165

BAX K53 clashes with BHX Y5

BAX K136 + E56 clashes with BHX R73

BAX F163 clashes with BHX F145

BAX L199 clashes with BHX W42

15 BAX M28 clashes with BHX W6

Analysing the three dimensional structure of the Bacillus agaradherens xylanase (BAX), having the X-ray structure 1QH7, and the three dimensional structure model of Paenibacillus sp. xylanase (PSX) having the X-ray structure 1BVV (also disclosing the amino acid sequence), it can be found that several "structural stop codons" can be found. Focusing mostly on the core "structural stop codons" the following residues can found to be important to mutate:

BAX R49 clashes with PSX Y166 + Q7

BAX K53 clashes with PSX Y5

BAX L199 clashes with PSX W42

BAX F163 clashes with PSX F146

25 BAX M28 clashes with PSX W6

BAX Y195 clashes with PSX N54.

CLAIMS

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- 1. A method of constructing a polypeptide, comprising:
 - a) selecting at least two parent polypeptides each having an amino acid sequence and a three-dimensional structure,
 - b) structurally aligning the three-dimensional structures, thereby aligning amino acid residues from different sequences,
 - c) selecting a first amino acid residue from one structure and a second residue from another structure, such that:
 - i) the two residues are not aligned with each other in the structural alignment,
 - ii) a non-hydrogen atom of the first residue and a non-hydrogen atom of the second residue are located less than 2.7 Å apart, and
 - iii) each of the two residues is not Glycine and has a side chain having less than 30 % solvent accessibility, and
 - d) substituting or deleting the first and/or the second residue with a smaller residue, and
 - e) recombining the amino acid sequences after the substitution, and
 - f) preparing a DNA-sequence encoding the polypeptide of step e) and expressing the polypeptide in a transformed host organism.
- 2. The method of claim 1, wherein each pair of parent polypeptides has an amino acid identity of at least 50 %,
 - 3. The method of claim 1, wherein each pair of parent polypeptides has a homology of at least 50%.
 - 4. The method of any preceding claim, further comprising
 - a) superimposing the structures so as to align each non-hydrogen atom located < 10 Å of an atom in the first or the second residue, and
 - b) selecting two residues that are less than 1.5 Å apart in the new superimposition.
 - 5. The method of any preceding claim wherein the two selected residues after the substitution can form a hydrogen bond, a salt bridge or a cysteine bridge.
- 6. The method of any preceding claim wherein a non-hydrogen atom of the first residue and a non-hydrogen atom of the second residue are located less than 1.7 Å apart, particularly less than 1.5 Å, 1.2 Å, 1.1 Å or 1.0 Å apart.

7. The method of claim 1 wherein each parent polypeptide is an enzyme having an active site, and the structural alignment is done so as to align each non-hydrogen atom of the amino acid residues of the active sites

- 8. The method of the preceding claim wherein the enzymes belong to glycosyl hydrolase fam-5 ily 13, particularly comprising a cyclodextrin glucanotransferase and a maltogenic alphaamylase.
 - 9. The method of claim 6 or 7 which further comprises producing a polypeptide having the recombined amino acid sequence, testing the polypeptide for an enzymatic activity and selecting an enzymatically active polypeptide.
- 10. A polypeptide which has an amino acid identity of at least 80% to SEQ ID NO: 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25.
 - 11. A polynucleotide encoding any polypeptide of the above claim.
 - 12. A polypeptide which:

- a) has an amino acid sequence which is a hybrid of a maltogenic alpha-amylase and a cyclodextrin glucanotransferase,
 - b) has a smaller residue at a position corresponding to:
 - i) D209, L261, D267, M307, H503, T509, V626, K651 of SEQ ID NO: 6 or
 - ii) K7, Y181, N266, K270, L286, Y574, P592, S676 of SEQ ID NO: 17, and
 - c) has hydrolytic activity on starch.
- 20 13. A dough comprising the polypeptide of the preceding claim.

1					50
SEQ ID NO: 1	MSKKTLKRLL	ALVVVLFILS	GSGILDFSIT	SANAQQATDR	SNSVNYSTDG
SEQ ID NO: 2				SASAQQATDR	
SEQ ID NO: 3				PAWASPDTSV	
SEQ ID NO: 4				PVWASPDTSV	
SEO ID NO: 5				ASDTAV	
SEQ ID NO: 6				APDTSV	
SEQ ID NO: 7				AVYADPDTAV	
SEQ ID NO: 8				AIYADADTAV	
SEO ID NO: 9				PVHAAPDTSV	
SEQ ID NO: 10				PVHAAPDTSV	
SEQ ID NO: 10				PAQAAPDTSV	
SEQ ID NO: 12				APDTSV	
SEQ ID NO: 12				.LPTAAQADV	
SEQ ID NO: 13				.LPTVAEADV	
SEQ ID NO: 15				QKVTVEAAGN	
SEQ ID NO: 15				TIAAEPEETY	
PEG ID MO: 10	MKKNKFF	MISAATAISI	ALMIFFCSMQ	ITAMEPEETI	DFRREI
51					100
SEQ ID NO: 1	IYOIVTDRFY	DGDESNNPSG	ELYSEGCKNL	RKYCGGDWQG	IIDKIDDGYL
SEQ ID NO: 2				RKYCGGDWQG	
SEQ ID NO: 3				KLYFGGDWQG	
SEO ID NO: 4				KLYFGGDWQG	
SEQ ID NO: 5				KKYFGGDWQG	
SEQ ID NO: 6				KKYFGGDWQG	
SEQ ID NO: 7				KLYCGGDWQG	
SEQ ID NO: 8				KLYCGGDWQG	
SEQ ID NO: 9				RLYCGGDWQG	
SEQ ID NO: 10				RLYCGGDWQG	
SEQ ID NO: 11				RLYCGGDWQG	
SEQ ID NO: 12				RLYCGGDWQG	
SEQ ID NO: 13				HKYCGGDWQG	
SEQ ID NO: 14				HKYCGGDWQG	
SEQ ID NO: 15				RKYCGGDWQG	
SEQ ID NO: 16				KKYTGGDLRG	
DIQ ID NO. 10	TIPETEDATE	DODI BINIMAGI	NOMITOINNE	ICCTTOODERG	TINKTII
10:	1				150
SEQ ID NO: 1	TNMGVTALWI	SPPVENIFET	IDDESGTT	SYHGYWARDY	KKTNPFFGST
SEQ ID NO: 2	TNMGVTALWI	SPPVENIFET	IDDEFGTT	SYHGYWARDY	KKTNPFFGST
SEQ ID NO: 3	TGMGVTALWI	SQPVENITSV	IKYSGVNN.T	SYHGYWARDF	KQTNDAFGDF
SEQ ID NO: 4	TGMGITALWI	SQPVENITAV	INYSGVNN.T	AYHGYWPRDF	KKTNAAFGSF
SEQ ID NO: 5	TGMGVTAIWI	SQPVENIYAV	LPDSTFGGST	SYHGYWARDF	KRTNPYFGSF
SEQ ID NO: 6	TGMGITAIWI	SQPVENIYAV	LPDSTFGGST	SYHGYWARDF	KKTNPFFGSF
SEQ ID NO: 7	SDLGVTALWI	SQPVENIFAT	INYSGVTN.T	AYHGYWARDF	KKTNPYFGTM
SEQ ID NO: 8	SDLGVTALWI	SQPVENIFAT	INYSGVTN.T	AYHGYWARDF	KKTNPYFGTM
SEQ ID NO: 9	TGMGITAIWI	SQPVENIYSV	INYSGVNN.T	AYHGYWARDF	KKTNPAYGTM
SEQ ID NO: 10				AYHGYWARDF	
SEQ ID NO: 11				AYHGYWARDF	
SEQ ID NO: 12				AYHGYWARDF	
SEQ ID NO: 13				SYHGYWARDY	
SEQ ID NO: 14				SYHGYWARDY	
SEQ ID NO: 15				SYHGYWARDF	
SEQ ID NO: 16		TPPIDNV		GYHGYWGRDY	
		-		_	· · · -

Fig. 1

151					200
SEQ ID NO: 1	EDFERLIETA	HSH. DIKTV	TDLAPNHTSP	ADFDNPNYAE	NGILYDNGNY
SEQ ID NO: 2		HSHDIKIV	TDLAPNHTSP	ADFDNPDYAE	NGVLYDDGNY
SEQ ID NO: 3		HAHNIKVV		ADRDNPGFAE	
SEQ ID NO: 4	-	HSHNIKVV		ASSTDPSFAE	
SEQ ID NO: 5		HAHNIKVI		ASETDPTYAE	
SEQ ID NO: 6		HAHNIKVI		ASETDPTYGE	
SEQ ID NO: 7		HAKGIKIV		AMETDTSFAE	
SEQ ID NO: 7		HAKGIKII		AMETDISFAE	
SEQ ID NO: 8		HAHNIKVI		ASSDDPSFAE	
SEQ ID NO: 9	~	HAHNIKVI		ASSDDPSFAE	
_		HAKNIKVI		ASSDOPSFAE	
SEQ ID NO: 11		HAKNIKVI		ASLDOPSFAE	
SEQ ID NO: 12					
SEQ ID NO: 13				ALETDPSYAE	
SEQ ID NO: 14				ALETNPNYVE	
SEQ ID NO: 15		HAKGIKVI		ASETNPSYME	
SEQ ID NO: 16	DDFKELTSLM	HSPDYNMKLV	LDYAPNHSNA	NDENE	FGALYRDGVF
20	1				250
SEQ ID NO: 1		DLFLYNGG	THE CHVETE	IYRNLFDLAS	
SEQ ID NO: 1	LGSYSDDS			IYRNLFDLAS	
~	LGAYSNDTA.			IYKNLYDLAD	
~ ~				IYKNLYDLAD	INONNNTIDS
SEQ ID NO: 4	LGKYSNDTA.			IYRNLFDLAD	~
SEQ ID NO: 5	LGGYTNDTN.	GYFHHYGG			~~
SEQ ID NO: 6	LGGYTNDTN.	GYFHHYGG		IYRNLFDLAD	
SEQ ID NO: 7	VGGYTNDTN.	GYFHHNGG		IYKNLYDLAD	
SEQ ID NO: 8	VGGYTNDTN.	GYFHHNGG		IYKNLYDLAD	
SEQ ID NO: 9	LGGYTNDTQ.	NLFHHYGG		IYKNLYDLAD	
SEQ ID NO: 10	LGGYTNDTQ.			IYKNLYDLAD	
SEQ ID NO: 11	LGGYTNDTQ.	NLFHHNGG		IYKNLYDLAD	
SEQ ID NO: 12		NLFHHNGG		IYKNLYDLAD	
SEQ ID NO: 13		NLFHHNGG		IYRNLYDLAD	-
SEQ ID NO: 14	LGNYSNDQQ.	NLFHHNGG		IYRNLYDLAD	
SEQ ID NO: 15	LGGYTNDAN.	MYFHHNGG	.TTFSSLEDG	IYRNLFDLAD	LNHQNPVIDR
SEQ ID NO: 16	ITDYPTNVAA	NTGWYHHNGG	VTNWNDFFQV	KNHNLFNLSD	LNQSNTDVYQ
25			1 1 1		300
SEQ ID NO: 1				KAYMDTIY.D	
SEQ ID NO: 2				KAYMDTIY.D	
SEQ ID NO: 3				KSFVSSIYGG	
SEQ ID NO: 4				KSYVSSIYSS	
SEQ ID NO: 5				KNFMDSIL.S	
SEQ ID NO: 6	YLKAAIKLWL	DMGIDGIRMD	AVKHMAFGWQ	KNFMDSIL.S	YRPVF
SEQ ID NO: 7				KSWMSSIY.A	
SEQ ID NO: 8	YFKDAIKLWL	DMGVDGIRVD	AVKHMPQGWQ	KNWMSSIY.A	HKPVF
SEQ ID NO: 9	YLKDAIKMWL	DLGVDGIRVD	AVKHMPFGWQ	KSFMATIN.N	YKPVF
SEQ ID NO: 10	YLKDAIKMWL	DLGVDGIRVD	AVKHMPFGWQ	KSFMSTIN.N	YKPVF
SEQ ID NO: 11				KSFMAAVN.N	
SEQ ID NO: 12				KSFMATVN.N	
SEQ ID NO: 13				TSLMSDIY.A	
SEQ ID NO: 14				TSLMSEIY.S	
SEQ ID NO: 15				KSLMDEID.N	
SEQ ID NO: 16				QKWTSDIYDY	
DEG LD NO. 10				******	

Fig. 1 continued

SEQ ID NO: 1 TFGEWFTGPYG.NEDY TKFANNSGMS VLDFRFAQTT RNVIGNNE SEQ ID NO: 2 TFGEWFTGPSG.NEDY TKFANNSGMS VLDFRFAQTT RNVIGNNE SEQ ID NO: 3 TFGEWYLGADQTDGDN IKFANESGMN LLDFEYAQEV REVFRDKS SEQ ID NO: 4 TFGEWFLGPDEMTQDN INFANQSGMH LLDFAFAQEI REVFRDKS SEQ ID NO: 5 TFGEWFLGTNEIDVNN TYFANESGMS LLDFRFSQKV RQVFRDNS SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDNS SEQ ID NO: 7 TFGEWFLGSAASDADN TDFANKSGMS LLDFRFNSAV RNVFRDNS	
SEQ ID NO: 2 TFGEWFTGPSG.NEDY TKFANNSGMS VLDFRFAQTT RNVIGNNY SEQ ID NO: 3 TFGEWYLGADQTDGDN IKFANESGMN LLDFEYAQEV REVFRDKY SEQ ID NO: 4 TFGEWFLGPDEMTQDN INFANQSGMH LLDFAFAQEI REVFRDKY SEQ ID NO: 5 TFGEWFLGTNEIDVNN TYFANESGMS LLDFRFSQKV RQVFRDNY SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDNY	1GT
SEQ ID NO: 3 TFGEWYLGADQTDGDN IKFANESGMN LLDFEYAQEV REVFRDKS SEQ ID NO: 4 TFGEWFLGPDEMTQDN INFANQSGMH LLDFAFAQEI REVFRDKS SEQ ID NO: 5 TFGEWFLGTNEIDVNN TYFANESGMS LLDFRFSQKV RQVFRDNS SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDNS	
SEQ ID NO: 5 TFGEWFLGTNEIDVNN TYFANESGMS LLDFRFSQKV RQVFRDN SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDN	ľET
SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDN	ET
SEQ ID NO: 6 TFGEWYLGTNEVDPNN TYFANESGMS LLDFRFAQKV RQVFRDN	
	rsn
SEQ ID NO: 8 TFGEWFLGSAAPDADN TDFANESGMS LLDFRFNSAV RNVFRDN	rsn
SEQ ID NO: 9 TFGEWFLGVNEISPEY HQFANESGMS LLDFRFAQKA RQVFRDN	CDN
SEQ ID NO: 10 NFGEWFLGVNEISPEY HQFANESGMS LLDFPFAQKA RQVFRDN	Γ DN
SEQ ID NO: 11 TFGEWFLGVNEVSPEN HKFANESGMS LLDFRFAQKV RQVFRDN	CDN
SEQ ID NO: 12 TFGEWFLGVNEVSAEN HKFANVSGMS LLDFRFAQKV RQVFKDN	LDN
SEQ ID NO: 13 TFGEWFLGSGEVDPQN HHFANESGMS LLDFQFGQTI RDVLMDGS	SSN
SEQ ID NO: 14 TFGEWFLGSGEVDPQN HHFANESGMS LLDFQFGQTI RNVLKDR	CSN
SEQ ID NO: 15 TFGEWFLSENEVDANN HYFANESGMS LLDFRFGQKL RQVLRNNS	
SEQ ID NO: 16 FFGEWFGASA NTTTGVDGNA IDYANTSGSA LLDFGFRDTL ERVLVGRS	3GN
351 400	
SEQ ID NO: 1 .MYDIEKMLT DTENDYDRPQ DQVTFLDNHD MSRFTNDGES T	
SEQ ID NO: 2 .MYDIEKMLT DTENDYDRPQ DQVTFLDNHD MSRFTNGGES T	
SEQ ID NO: 3 .MKDLYEVLA STESQYDYIN NMVTFIDNHD MDRFQVAGSG T	
SEQ ID NO: 4 .MTDLNSVIS STGSSYNYIN NMVTFIDNHD MDRFQQAGAS T	
SEQ ID NO: 5 .MYGLDSMIQ STASDYNFIN DMVTFIDNHD MDRFYNG.GS T	
SEQ ID NO: 6 .MYGLDSMIQ STAADYNFIN DMVTFIDNHD MDRFYTG.GS T	
SEQ ID NO: 7 .MYALDSMIN STATDYNQVN DQVTFIDNHD MDRFKTSAVN N	
SEQ ID NO: 8 .MYALDSMLT ATAADYNQVN DQVTFIDNHD MDRFKTSAVN N	
SEQ ID NO: 9 .MYGLKAMLE GSEVDYAQVN DQVTFIDNHD MERFHTSNGD R	
SEQ ID NO: 10 .MYGLKAMLE GSEVDYAQVN DQVTFIDNHD MERFHTSNGD R	
SEQ ID NO: 11 .MYGLKAMLE GSAADYAQVD DQVTFIDNHD MERFHASNAN R	
SEQ ID NO: 12 .MYGLKSMLE GSATDYAQME DQVTFIDNHD MERFHNNSAN R	
SEQ ID NO: 13 .WYDFNEMIA STEEDYDEVI DQVTFIDNHD MSRFSFEQSS N	
SEQ ID NO: 14 .WYDFNEMIT STEKEYNEVI DQVTFIDNHD MSRFSVGSSS N	
SEQ ID NO: 15 .WYGFNQMIQ DTASAYDEVL DQVTFIDNHD MDRFMIDGGD P	
SEQ ID NO: 16 TMKTLNSYLI KRQTVFTSDD WQVVFMDNHD MARIGTALRS NATTFGPO	
-	
401 450	
SEQ ID NO: 1RTTDIGLA LMLTSRGVPT IYYGTEQYME G	
SEQ ID NO: 2RTTDIGLA LMLTSRGVPT IYYGTEQYMK G	
SEQ ID NO: 3RATEQALA LTLTSRGVPA IYYGTEQYMT G	
SEQ ID NO: 4 RPTEQALA VTLTSRGVPA IYYGTEQYMT G	
SEQ ID NO: 5 RPVEQALA FTLTSRGVPA IYYGTEQYMT G	
SEQ ID NO: 6	
SEQ ID NO: 7	
SEQ ID NO: 8	
SEQ ID NO: 9 RKLEQALA FTLTSRGVPA IYYGSEQYMS G	
SEQ ID NO: 10	
SEQ ID NO: 11	
SEQ ID NO: 12 RKLEQALA FTLTSRGVPA IYYGTEQYMS G	
SEQ ID NO: 13 RHTDIALA VLLTSRGVPT IYYGTEQYLT G	
SEQ ID NO: 14 RQTDMALA VLLTSRGVPT IYYGTEQYVT G	
SEQ ID NO: 15RKVDMALA VLLTSRGVPN IYYGTEQYMT G	

Fig. 1 continued

45:	1				500
SEQ ID NO: 1	DGDPGSRGMM	ESFGENTDAY	KLIQKLAPLR	KSNPAYGYGT	TKERWINDDV
SEQ ID NO: 2				KSNPAYGYGT	
SEQ ID NO: 3				KSNPAIAYGT	
SEO ID NO: 4	NGDPNNRGMM				TTQRWVNSDV
SEQ ID NO: 5	NGDPYNRAMM				TQQRWINNDV
SEQ ID NO: 6	NGDPYNRAMM				QKQRWINNDV
SEQ ID NO: 7	NGDPDNRAKM	PSFSKSTTAF	NVISKLAPLR	KSNPAIAYGS	TQQRWINNDV
SEQ ID NO: 8	NGDPDNRGKM				TQQRWINNDV
SEQ ID NO: 9	GNDPDNRARL	PSFSTTTTAY	QVIQKLAPLR	KSNPAIAYGS	THERWINNDV
SEQ ID NO: 10			QVIQKLAPLR		TOERWINNDV
SEQ ID NO: 11			QVIQKLAPLR		TOERWINNDV
SEQ ID NO: 12			QVSKKLAPLR		TOERWINNDV
SEO ID NO: 13				QNNPALGYGN	TSERWINSDV
SEQ ID NO: 14			QIISKLASLR		TTERWLNEDI
SEQ ID NO: 15			QVIQKLSSLR		TEQRWINGDV
SEQ ID NO: 16				KSSPAIQNGT	
PEG ID NO. IO	CDD1 11VICD1G1				
501				550	
SEO ID NO: 1	IIYERNFGDN	YALIAINRNL	NTSYNIOGLO	TEMPSNSYDD	VLDGLLDGQS
SEQ ID NO: 2				TEMPSNSYDD	
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SEQ ID NO: 4				TALPNGTYTD	
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Fig. 1 continued

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SEQ ID NO: 9 DGRGFGSGKG TVYFGTTAVT GADIVAWEDT QIQVKIPAVP GGIYDIRVAN
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Fig. 1 continued

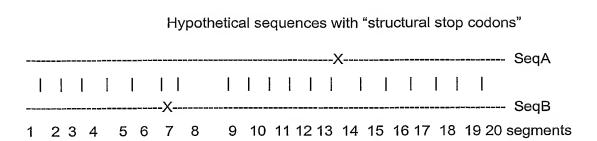
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ID	NO:	16	QSGANNQFNS	NDTQTTNGSF	
	ID	ID NO:	ID NO: 1 ID NO: 2 ID NO: 3 ID NO: 4 ID NO: 5 ID NO: 6 ID NO: 7 ID NO: 8 ID NO: 9 ID NO: 10 ID NO: 11 ID NO: 12 ID NO: 13 ID NO: 14 ID NO: 15	ID NO: 2 QSGANQTYSS ID NO: 3 EGGGNHTYTT ID NO: 4 EGGNNHTFTS ID NO: 5 EGGSNHTYTV ID NO: 6 EGGYNHVYTT ID NO: 7 ESGSNHTFTT ID NO: 8 EGGSNHTFTT ID NO: 9 EGGANRTFTT ID NO: 10 EGGANRTFTT ID NO: 11 EGGSNHTFTA ID NO: 12 EGGSNHTFTA ID NO: 13 ESGNHTYTT ID NO: 14 QSGNNRTYTS ID NO: 15 ESGSNHVYTT	ID NO: 1 QSGANHTYSS PESGTGIIRV ID NO: 2 QSGANQTYSS PESGTGIIRV ID NO: 3 EGGGNHTYTT PASGVGTVTV ID NO: 4 EGGNNHTFTS PSSGVATVTV ID NO: 5 EGGSNHTYTV PSSSTGTVIV ID NO: 6 EGGYNHVYTT PTSGTATVIV ID NO: 7 ESGSNHTFTT PASGTATVTV ID NO: 8 EGGSNHTFTT PTSGTATVTI ID NO: 9 EGGANRTFTT PTSGTATVNV ID NO: 10 EGGANRTFTT PTSGTATVNV ID NO: 11 EGGSNHTFTA PTSGTATIVV ID NO: 12 EGGSNHTFTA PTSGTATIVV ID NO: 13 ESGNHTFTA PTSGTATIVV ID NO: 14 QSGNNRTYTS PTTGTDTVMI ID NO: 15 ESGSNHVYTT PTNTTGKIIV

Fig. 1 continued

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QLG\ GMG\	TTIWLSPVLDI TAIWISQPVEI	NLDTLAGT NIYAVLPDSTFO	DNTGYHGYWI GSTSYHGYW	RDFKQIEEH ARDFKRTNPY	FGNWTTFDTL FGSFTDFQNL	VNDAHQNGIKVI INTAHAHNIKVI	IDF	128 137
VPNE	ISTPFKANDST	FAEGGALYNNG:	TYMGNYFDDAT	rkgyfhhngd	* ISNWDDRYEA	QWKNFTDPAGFS	SLAD	198
APNI	HTSPASETDPT	YAENGRLYDNGT	TLLGGYTNDT-	-NGYFHHYGG	T-DFSSYEDG	IYRNLFI	LAD	200
LSQE	ENGTIAQYLTD	AAVQLVAHGADO	LRIDAVKHFI	NSGFSKSLAD	KLYQKKDIFI	VGEWYGDD-PGT FGEWFLG-TNE	* CANH	267 267
ĿΝQζ	* SN2.1.TD2.1.FK2	* *	STKUDA (KUMI	SE GMÖVNEMD	PINPIKEALI	* *	*	207
* 733.1	₹₽₩₩₽₽₩₩₽₽₩₩	* UDFDT,NTVTRNI	/FGTFTOTMYI	OLNNMVNOTG	* NEYKYKENLI	TFIDNHDMSRFI	LSVN	337
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SMK	.TT¶&,7∆∩H.TM <i>L</i>	* TSRGTPSTVYG	reoymaggnd!	PYNRGMMPAF	DTTTTAFKEV	STLAGLRRNNA <i>I</i>	YIQY	407
GSTI	RPVEQALAFTL'	TSRGVPAIYYG	reqymtgngdi	PYNRAMMTSF	NTSTTAYNVI	KKLAPLRKSNPA	YAIAY	406
* GTT	rqrwinndvyi	YERKFFNDVVL	/AINRNTQSS	YSISGLQTAL	PNGSYADYLS	GLLGGNGISVS	-NGS	476
GTT	QQRWINNDVY1	YERKFGNNVAL	/AINRNLSTS	YNTTGLYTAL	PAGTATOARG	GLLNGNSISVA	*	4/6
VASI VTPI	FTLAPGAVSVW FTLSAGEVAVW	QYST-SASAPQ QYVSSSN-SPL	IGSVAPNMGII IGHVGPTMTK	PGNVVTIDGK AGQTITIDGR	GFGTTQGTVI GFGTTSGQVI	FGGVTATVKSW: FGSTAGTIVSWI	FSNR ODTE	545 545
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							*	
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Fig. 2

8/8



Hypothetical sequences with "structural stop codons"

Fig. 3

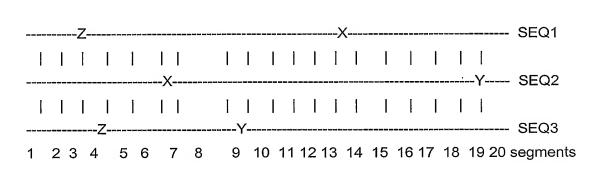


Fig. 4

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Asp Gly Ile Tyr Gln Ile Val Thr Asp Arg Phe Tyr Asp Gly Asp Glu 50 60

Ser Asn Asn Pro Ser Gly Glu Leu Tyr Ser Glu Gly Cys Lys Asn Leu 65 70 75 80

Arg Lys Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asp Lys Ile Asp 85 90 95

Asp Gly Tyr Leu Thr Asn Met Gly Val Thr Ala Leu Trp Ile Ser Pro 100 105 110

Pro Val Glu Asn Ile Phe Glu Thr Ile Asp Asp Glu Ser Gly Thr Thr 115 120 125

Ser Tyr His Gly Tyr Trp Ala Arg Asp Tyr Lys Lys Thr Asn Pro Phe 130 140

Phe Gly Ser Thr Glu Asp Phe Glu Arg Leu Ile Glu Thr Ala His Ser 145 150 155 160

His Asp Ile Lys Ile Val Ile Asp Leu Ala Pro Asn His Thr Ser Pro 165 170 175

Ala Asp Phe Asp Asn Pro Asn Tyr Ala Glu Asn Gly Ile Leu Tyr Asp 180 185 190

Asn Gly Asn Tyr Val Ser Ser Tyr Ser Asp Asn Ser Asp Leu Phe Leu Page 1

195 200 205

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Gly Gln Ser Ile Val Val Asp Asn Gly Glu Val Asn Glu Phe Gln
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Met Ser Pro Gly Glu Val Gly Val Trp Glu Phe Glu Ala Thr Asn Val 515 520 525

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Arg Thr Val Thr Ile Ser Gly Glu Gly Phe Gly Ser Ser Pro Gly Thr 545 550 555

Val Gln Phe Gly Ser Thr Ser Ala Glu Ile Val Ser Trp Asn Asp Thr 565 570 575

Val Ile Ile Ile Thr Val Pro Asn Asn Glu Ala Gly Tyr His Asp Ile 580 585 590

Thr Val Val Thr Glu Asp Glu Gln Val Ser Asn Ala Tyr Glu Phe Glu 595 600 605

Val Leu Thr Ala Asp Gln Val Thr Val Arg Phe Ile Ile Asp Asn Ala 610 615 620

Glu Thr Lys Met Gly Glu Asn Ile Phe Leu Val Gly Asn Val His Glu 625 630 635 640

Leu Gly Asn Trp Asp Pro Glu Gln Ser Val Gly Arg Phe Phe Asn Gln 645 650

Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Asn Val Pro Ala 660 665 670

Asn Thr Asp Leu Glu Phe Lys Phe Ile Lys Ile Asp Gln Asp Asn Asn 675 680 685

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Bacillus agaradherens

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Arg Thr Val Thr Ile Ser Gly Glu Gly Phe Gly Ser Ser Gln Gly Thr 545 550 555 Val His Phe Gly Ser Thr Ser Ala Glu Ile Leu Ser Trp Asn Asp Thr 565 570 575 Ile Ile Thr Leu Thr Val Pro Asn Asn Glu Ala Gly Tyr His Asp Ile 580 585 590 Thr Val Val Thr Glu Asp Glu Gln Val Ser Asn Ala Tyr Glu Phe Glu 595 600 605 Val Leu Thr Ala Asp Gln Val Thr Val Arg Phe Ile Ile Asp Asn Ala 610 615 620 Glu Thr Lys Leu Gly Glu Asn Val Phe Leu Val Gly Asn Val His Glu 625 630 635 640 Leu Gly Asn Trp Asp Pro Glu Gln Ser Val Gly Arg Phe Phe Asn Gln 645 650 655 Ile Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Asn Val Pro Ala 660 665 670 Asn Thr Asp Leu Glu Phe Lys Phe Ile Lys Ile Asp Gln Asp Asn Asn 675 680 685 Val Ile Trp Gln Ser Gly Ala Asn Gln Thr Tyr Ser Ser Pro Glu Ser 690 695 700 Gly Thr Gly Ile Ile Arg Val Asp Trp 705

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Met Ala Leu Gly Ile Ser Leu Pro Ala Trp Ala Ser Pro Asp Thr Ser 20 25 30

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Panibacillus macerans

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Leu Gly Ser Trp Asp Pro Asn Lys Ala Ile Gly Pro Met Tyr Asn Gln 645 650 655

Val Ile Ala Lys Tyr Pro Ser Trp Tyr Tyr Asp Val Ser Val Pro Ala 660 665 670

Gly Thr Lys Leu Asp Phe Lys Phe Ile Lys Lys Gly Gly Gly Thr Val 675 680 685

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35 40 45

Thr Asp Arg Phe Val Asp Gly Asn Ser Ala Asn Asn Pro Thr Gly Ala 50 60

Ala Phe Ser Ser Asp His Ser Asn Leu Lys Leu Tyr Phe Gly Gly Asp 65 70 75 80

Trp Gln Gly Ile Thr Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met 85 90 95

Gly Ile Thr Ala Leu Trp Ile Ser Gln Pro Val Glu Asn Ile Thr Ala 100 105 110

Val Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala Tyr His Gly Tyr Trp 115 120 125

Pro Arg Asp Phe Lys Lys Thr Asn Ala Ala Phe Gly Ser Phe Thr Asp 130 135 140 Phe Ser Asn Leu Ile Ala Ala Ala His Ser His Asn Ile Lys Val Val 145 150 155 160 Met Asp Phe Ala Pro Asn His Thr Asn Pro Ala Ser Ser Thr Asp Pro 165 170 175 Ser Phe Ala Glu Asn Gly Ala Leu Tyr Asn Asn Gly Thr Leu Leu Gly 180 185 190 Lys Tyr Ser Asn Asp Thr Ala Gly Leu Phe His His Asn Gly Gly Thr 195 200 205 Asp Phe Ser Thr Thr Glu Ser Gly Ile Tyr Lys Asn Leu Tyr Asp Leu 210 215 220 Ala Asp Ile Asn Gln Asn Asn Asn Thr Ile Asp Ser Tyr Leu Lys Glu 225 230 235 240 Ser Ile Gln Leu Trp Leu Asn Leu Gly Val Asp Gly Ile Arg Phe Asp 245 250 255 Ala Val Lys His Met Pro Gln Gly Trp Gln Lys Ser Tyr Val Ser Ser 260 265 270 Ile Tyr Ser Ser Ala Asn Pro Val Phe Thr Phe Gly Glu Trp Phe Leu 275 280 285 Gly Pro Asp Glu Met Thr Gln Asp Asn Ile Asn Phe Ala Asn Gln Ser 290 295 300 Gly Met His Leu Leu Asp Phe Ala Phe Ala Gln Glu Ile Arg Glu Val 305 310 315 320 Phe Arg Asp Lys Ser Glu Thr Met Thr Asp Leu Asn Ser Val Ile Ser 325 330 335 Ser Thr Gly Ser Ser Tyr Asn Tyr Ile Asn Asn Met Val Thr Phe Ile 340 345 350 Asp Asn His Asp Met Asp Arg Phe Gln Gln Ala Gly Ala Ser Thr Arg 355 360 365 Pro Thr Glu Gln Ala Leu Ala Val Thr Leu Thr Ser Arg Gly Val Pro 370 375 380 Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly Asn Gly Asp Pro 385 390 395 400

Asn Asn Arg Gly Met Met Thr Gly Phe Asp Thr Asn Lys Thr Ala Tyr 405 410 415 Lys Val Ile Lys Ala Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Leu 420 425 430 Ala Tyr Gly Ser Thr Thr Gln Arg Trp Val Asn Ser Asp Val Tyr Val 435 440 445 Tyr Glu Arg Lys Phe Gly Ser Asn Val Ala Leu Val Ala Val Asn Arg 450 455 460 Ser Ser Thr Thr Ala Tyr Pro Ile Ser Gly Ala Leu Thr Ala Leu Pro 465 470 475 480 Asn Gly Thr Tyr Thr Asp Val Leu Gly Gly Leu Leu Asn Gly Asn Ser 485 490 495 Ile Thr Val Asn Gly Gly Thr Val Ser Asn Phe Thr Leu Ala Ala Gly 500 510 Gly Thr Ala Val Trp Gln Tyr Thr Thr Glu Ser Ser Pro Ile Ile 515 520 Gly Asn Val Gly Pro Thr Met Gly Lys Pro Gly Asn Thr Ile Thr Ile 530 540 Asp Gly Arg Gly Phe Gly Thr Thr Lys Asn Lys Val Thr Phe Gly Thr 545 550 555 Thr Ala Val Thr Gly Ala Asn Ile Val Ser Trp Glu Asp Thr Glu Ile 565 570 575 Lys Val Lys Val Pro Asn Val Ala Ala Gly Asn Thr Ala Val Thr Val 580 585 Thr Asn Ala Ala Gly Thr Thr Ser Ala Ala Phe Asn Asn Phe Asn Val 595 600 605 Leu Thr Ala Asp Gln Val Thr Val Arg Phe Lys Val Asn Asn Ala Thr 610 620 Thr Ala Leu Gly Gln Asn Val Tyr Leu Thr Gly Asn Val Ala Glu Leu 625 630 635 640 Gly Asn Trp Thr Ala Ala Asn Ala Ile Gly Pro Met Tyr Asn Gln Val 645 650 655 Glu Ala Ser Tyr Pro Thr Trp Tyr Phe Asp Val Ser Val Pro Ala Asn 660 665 670

Thr Ala Leu Gln Phe Lys Phe Ile Lys Val Asn Gly Ser Thr Val Thr 675 680 685

Trp Glu Gly Gly Asn Asn His Thr Phe Thr Ser Pro Ser Ser Gly Val 690 695 700

Ala Thr Val Thr Val Asp Trp Gln Asn 705

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<211> 683 <212> PRT

<213> Thermoanaerobacterium thermosulfurigenes

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Ala Ser Asp Thr Ala Val Ser Asn Val Val Asn Tyr Ser Thr Asp Val 1 10 15

Ile Tyr Gln Ile Val Thr Asp Arg Phe Val Asp Gly Asn Thr Ser Asn 20 25 30

Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys 35 40 45

Tyr Phe Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly 50 60

Tyr Leu Thr Gly Met Gly Val Thr Ala Ile Trp Ile Ser Gln Pro Val 65 70 75 80

Glu Asn Ile Tyr Ala Val Leu Pro Asp Ser Thr Phe Gly Gly Ser Thr 85 90 95

Ser Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Arg Thr Asn Pro Tyr 100 105 110

Phe Gly Ser Phe Thr Asp Phe Gln Asn Leu Ile Asn Thr Ala His Ala 115 120 125

His Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro 130 135 140

Ala Ser Glu Thr Asp Pro Thr Tyr Ala Glu Asn Gly Arg Leu Tyr Asp 145 150 155 160

Asn Gly Thr Leu Leu Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr Phe 165 170 175

His His Tyr Gly Gly Thr Asp Phe Ser Ser Tyr Glu Asp Gly Ile Tyr 180 185

Arg Asn Leu Phe Asp Leu Ala Asp Leu Asn Gln Gln Asn Ser Thr Ile Page 12

195 200 205

Ser Tyr Leu Lys Ser Ala Ile Lys Val Trp Leu Asp Met Gly Ile 210 220 Asp Gly Ile Arg Leu Asp Ala Val Lys His Met Pro Phe Gly Trp Gln 225 230 235 240 Lys Asn Phe Met Asp Ser Ile Leu Ser Tyr Arg Pro Val Phe Thr Phe 245 250 255 Gly Glu Trp Phe Leu Gly Thr Asn Glu Ile Asp Val Asn Asn Thr Tyr 260 265 270 Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ser Gln 275 280 285 Lys Val Arg Gln Val Phe Arg Asp Asn Thr Asp Thr Met Tyr Gly Leu 290 295 300 Asp Ser Met Ile Gln Ser Thr Ala Ser Asp Tyr Asn Phe Ile Asn Asp 305 310 315 320 Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Asn Gly 325 330 335 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser 340 345 350 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly 355 360 365 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asn Thr Ser 370 380 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser 385 390 395 400 Asn Pro Ala Ile Ala Tyr Gly Thr Thr Gln Gln Arg Trp Ile Asn Asn 405 410 415 Asp Val Tyr Ile Tyr Glu Arg Lys Phe Gly Asn Asn Val Ala Leu Val 420 425 430 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Asn Ile Thr Gly Leu Tyr 435 440 445Thr Ala Leu Pro Ala Gly Thr Tyr Thr Asp Val Leu Gly Gly Leu Leu 450 460 Asn Gly Asn Ser Ile Ser Val Ala Ser Asp Gly Ser Val Thr Pro Phe Page 13

465 470 475 480

Thr Leu Ser Ala Gly Glu Val Ala Val Trp Gln Tyr Val Ser Ser Ser 485 490 495

Asn Ser Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly 500 510

Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ser Gly Gln 515 525

Val Leu Phe Gly Ser Thr Ala Gly Thr Ile Val Ser Trp Asp Asp Thr 530 540

Glu Val Lys Val Lys Val Pro Ser Val Thr Pro Gly Lys Tyr Asn Ile 545 550 555 560

Ser Leu Lys Thr Ser Ser Gly Ala Thr Ser Asn Thr Tyr Asn Asn Ile 565 570 575

Asn Ile Leu Thr Gly Asn Gln Ile Cys Val Arg Phe Val Val Asn Asn 580 585 590

Ala Ser Thr Val Tyr Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala 595 600 605

Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn 610 615 620

Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro 625 630 635 640

Ala Gly Thr Thr Ile Gln Phe Lys Phe Ile Lys Lys Asn Gly Asn Thr $645 \hspace{1.5cm} 650 \hspace{1.5cm} 655$

Ile Thr Trp Glu Gly Gly Ser Asn His Thr Tyr Thr Val Pro Ser Ser 660 665 670

Ser Thr Gly Thr Val Ile Val Asn Trp Gln Gln 675 680

<400> 6

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Ile Tyr Gln Ile Val Thr Asp Arg Phe Leu Asp Gly Asn Pro Ser Asn 20 25 30

<210> 6 <211> 683

<211> 683 <212> PRT

<213> Thermoanaerobacter sp.

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Asp Ser Met Ile Gln Ser Thr Ala Ala Asp Tyr Asn Phe Ile Asn Asp 305 310 315 Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Thr Gly 325 330 335 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser 340 345 350 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly 355 360 365 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asp Thr Thr 370 380 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser 385 390 395 400 Asn Pro Ala Ile Ala Tyr Gly Thr Gln Lys Gln Arg Trp Ile Asn Asn 405 410 415Asp Val Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu Val 420 425 430 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu Tyr 435 440 445 Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu Leu 450 460 Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro Phe 465 470 475 480 Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr Thr 485 490 495 Asn Pro Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly 500 510 Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly Gln 515 520 525 Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp Thr 530 540 Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn Ile 545 550 560 Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn Ile 565 570 575 Page 16

Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn Asn 580 585 Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala 595 600 605 Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn 610 615 620Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro 625 630 635 640 Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser Thr 645 650 655 Val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr Ser 660 665 670 Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro 675 680

Met Phe Gln Met Ala Lys Arg Ala Phe Leu Ser Thr Thr Leu Thr Leu 1 5 10 15

Gly Leu Leu Ala Gly Ser Ala Leu Pro Phe Leu Pro Ala Ser Ala Val 20 25 30

Tyr Ala Asp Pro Asp Thr Ala Val Thr Asn Lys Gln Ser Phe Ser Thr 35 40 45

Asp Val Ile Tyr Gln Val Phe Thr Asp Arg Phe Leu Asp Gly Asn Pro

Ser Asn Asn Pro Thr Gly Ala Ala Tyr Asp Ala Thr Cys Ser Asn Leu 70 75 80

Lys Leu Tyr Cys Gly Gly Asp Trp Gln Gly Leu Ile Asn Lys Ile Asn 85 90 95

Asp Asn Tyr Phe Ser Asp Leu Gly Val Thr Ala Leu Trp Ile Ser Gln
100 105 110

Pro Val Glu Asn Ile Phe Ala Thr Ile Asn Tyr Ser Gly Val Thr Asn 115 120 125

<210>

⁷ 718 <211><212>

PRT <213> Bacillus circulans

⁷ <400>

Thr Ala Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro 130 135 140 Tyr Phe Gly Thr Met Ala Asp Phe Gln Asn Leu Ile Thr Thr Ala His 145 150 155 160 Ala Lys Gly Ile Lys Ile Val Ile Asp Phe Ala Pro Asn His Thr Ser 165 170 175 Pro Ala Met Glu Thr Asp Thr Ser Phe Ala Glu Asn Gly Arg Leu Tyr 180 185 190 Asp Asn Gly Thr Leu Val Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr 195 200 205 Phe His His Asn Gly Gly Ser Asp Phe Ser Ser Leu Glu Asn Gly Ile 210 215 220 Tyr Lys Asn Leu Tyr Asp Leu Ala Asp Phe Asn His Asn Asn Ala Thr 225 230 240 Ile Asp Lys Tyr Phe Lys Asp Ala Ile Lys Leu Trp Leu Asp Met Gly 245 250 Val Asp Gly Ile Arg Val Asp Ala Val Lys His Met Pro Leu Gly Trp 260 265 270 Gln Lys Ser Trp Met Ser Ser Ile Tyr Ala His Lys Pro Val Phe Thr 275 280 285 Phe Gly Glu Trp Phe Leu Gly Ser Ala Ala Ser Asp Ala Asp Asn Thr 290 295 300 Asp Phe Ala Asn Lys Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Asn 305 310 315 Ser Ala Val Arg Asn Val Phe Arg Asp Asn Thr Ser Asn Met Tyr Ala 325 330 335 Leu Asp Ser Met Ile Asn Ser Thr Ala Thr Asp Tyr Asn Gln Val Asn 340 345 350Asp Gln Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Lys Thr 355 360 365 Ser Ala Val Asn Asn Arg Arg Leu Glu Gln Ala Leu Ala Phe Thr Leu 370 380 Thr Ser Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Leu 385 390 395 400

Thr Gly Asn Gly Asp Pro Asp Asn Arg Ala Lys Met Pro Ser Phe Ser 405 410 415 Lys Ser Thr Thr Ala Phe Asn Val Ile Ser Lys Leu Ala Pro Leu Arg 420 425 430 Lys Ser Asn Pro Ala Ile Ala Tyr Gly Ser Thr Gln Gln Arg Trp Ile 435 440 445 Asn Asn Asp Val Tyr Val Tyr Glu Arg Lys Phe Gly Lys Ser Val Ala 450 460 Val Val Ala Val Asn Arg Asn Leu Ser Thr Ser Ala Ser Ile Thr Gly 465 470 475 480 Leu Ser Thr Ser Leu Pro Thr Gly Ser Tyr Thr Asp Val Leu Gly Gly
485 490 495 Val Leu Asn Gly Asn Asn Ile Thr Ser Thr Asn Gly Ser Ile Asn Asn 500 505 510 Phe Thr Leu Ala Ala Gly Ala Thr Ala Val Trp Gln Tyr Thr Thr Ala 515 520 525 Glu Thr Thr Pro Thr Ile Gly His Val Gly Pro Val Met Gly Lys Pro 530 . 535 540 Gly Asn Val Val Thr Ile Asp Gly Arg Gly Phe Gly Ser Thr Lys Gly 545 550 555 560 Thr Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ala Ala Ile Thr Ser 565 570 575 Trp Glu Asp Thr Gln Ile Lys Val Thr Ile Pro Ser Val Ala Ala Gly
580 585 590 Asn Tyr Ala Val Lys Val Ala Ala Ser Gly Val Asn Ser Asn Ala Tyr 595 600 605 Asn Asn Phe Thr Ile Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val 610 620 Val Asn Asn Ala Ser Thr Thr Leu Gly Gln Asn Leu Tyr Leu Thr Gly 625 630 635 640 Asn Val Ala Glu Leu Gly Asn Trp Ser Thr Gly Ser Thr Ala Ile Gly 645 650 655 Pro Ala Phe Asn Gln Val Ile His Gln Tyr Pro Thr Trp Tyr Tyr Asp
660 665 670

Val Ser Val Pro Ala Gly Lys Gln Leu Glu Phe Lys Phe Phe Lys Lys 675 680 685

Asn Gly Ser Thr Ile Thr Trp Glu Ser Gly Ser Asn His Thr Phe Thr 690 695 700

Thr Pro Ala Ser Gly Thr Ala Thr Val Thr Val Asn Trp Gln 705 710 715

<210> <211> 8 718

Bacillus sp. 38-2

Met Phe Gln Met Ala Lys Arg Val Leu Leu Ser Thr Thr Leu Thr Phe 10 15

Ser Leu Leu Ala Gly Ser Ala Leu Pro Phe Leu Pro Ala Ser Ala Ile 20 25 30

Tyr Ala Asp Ala Asp Thr Ala Val Thr Asn Lys Gln Asn Phe Ser Thr 35 40 45

Asp Val Ile Tyr Gln Val Phe Thr Asp Arg Phe Leu Asp Gly Asn Pro 50 60

Ser Asn Asn Pro Thr Gly Ala Ala Phe Asp Gly Thr Cys Ser Asn Leu 70 75 80

Lys Leu Tyr Cys Gly Gly Asp Trp Gln Gly Leu Val Asn Lys Ile Asn 85 90 95

Asp Asn Tyr Phe Ser Asp Leu Gly Val Thr Ala Leu Trp Ile Ser Gln
100 105 110

Pro Val Glu Asn Ile Phe Ala Thr Ile Asn Tyr Ser Gly Val Thr Asn 115 120 . 125

Thr Ala Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro 130 135 140

Tyr Phe Gly Thr Met Thr Asp Phe Gln Asn Leu Val Thr Thr Ala His 145 150 155 160

Ala Lys Gly Ile Lys Ile Ile Ile Asp Phe Ala Pro Asn His Thr Ser 165 170 175

Pro Ala Met Glu Thr Asp Thr Ser Phe Ala Glu Asn Gly Lys Leu Tyr 180 185 190

Asp Asn Gly Asn Leu Val Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr 195 200 205 Phe His His Asn Gly Gly Ser Asp Phe Ser Thr Leu Glu Asn Gly Ile 210 215 220 Tyr Lys Asn Leu Tyr Asp Leu Ala Asp Leu Asn His Asn Asn Ser Thr 225 230 240 Ile Asp Thr Tyr Phe Lys Asp Ala Ile Lys Leu Trp Leu Asp Met Gly 245 250 Val Asp Gly Ile Arg Val Asp Ala Val Lys His Met Pro Gln Gly Trp 260 265 270 Gln Lys Asn Trp Met Ser Ser Ile Tyr Ala His Lys Pro Val Phe Thr 275 280 285 Phe Gly Glu Trp Phe Leu Gly Ser Ala Ala Pro Asp Ala Asp Asn Thr 290 300 Asp Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Asn 305 310 315 320 Ser Ala Val Arg Asn Val Phe Arg Asp Asn Thr Ser Asn Met Tyr Ala 325 330 335 Leu Asp Ser Met Leu Thr Ala Thr Ala Ala Asp Tyr Asn Gln Val Asn 340 345 350 Asp Gln Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Lys Thr 355 360 365 Ser Ala Val Asn Asn Arg Arg Leu Glu Gln Ala Leu Ala Phe Thr Leu 370 375 380 Thr Ser Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Leu 385 390 395 400 Thr Gly Asn Gly Asp Pro Asp Asn Arg Gly Lys Met Pro Ser Phe Ser 405 410 415Lys Ser Thr Thr Ala Phe Asn Val Ile Ser Lys Leu Ala Pro Leu Arg 420 425 430 Lys Ser Asn Pro Ala Ile Ala Tyr Gly Ser Thr Gln Gln Arg Trp Ile 435 440 445 Asn Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Gly Lys Ser Val Ala 450 455 460

Val Val Ala Val Asn Arg Asn Leu Thr Thr Pro Thr Ser Ile Thr Asn 465 470 475 Leu Asn Thr Ser Leu Pro Ser Gly Thr Tyr Thr Asp Val Leu Gly Gly 485 490 495 Val Leu Asn Gly Asn Asn Ile Thr Ser Ser Gly Gly Asn Ile Ser Ser 500 505 510 Phe Thr Leu Ala Ala Gly Ala Thr Ala Val Trp Gln Tyr Thr Ala Ser 515 520 525 Glu Thr Thr Pro Thr Ile Gly His Val Gly Pro Val Met Gly Lys Pro 530 535 540 Gly Asn Val Val Thr Ile Asp Gly Arg Gly Phe Gly Ser Ala Lys Gly 545 550 555 560 Thr Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ser Ala Ile Thr Ser 570 575 Trp Glu Asp Thr Gln Ile Lys Val Thr Ile Pro Pro Val Ala Gly Gly 580 585 590 Asp Tyr Ala Val Lys Val Ala Ala Asn Gly Val Asn Ser Asn Ala Tyr 595 600 605 Asn Asp Phe Thr Ile Leu Ser Gly Asp Gln Val Ser Val Arg Phe Val 610 620 Ile Asn Asn Ala Thr Thr Ala Leu Gly Glu Asn Ile Tyr Leu Thr Gly 625 630 640 Asn Val Ser Glu Leu Gly Asn Trp Thr Thr Gly Ala Ala Ser Ile Gly 645 650 655 Pro Ala Phe Asn Gln Val Ile His Ala Tyr Pro Thr Trp Tyr Tyr Asp 660 665 670 Val Ser Val Pro Ala Gly Lys Gln Leu Glu Phe Lys Phe Phe Lys Lys 675 680 685 Asn Gly Ala Thr Ile Thr Trp Glu Gly Gly Ser Asn His Thr Phe Thr 690 700 Thr Pro Thr Ser Gly Thr Ala Thr Val Thr Ile Asn Trp Gln 705 710 715

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<212> PRT <213> Bacillus sp. 1011

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260 265 270

Ile Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly Glu Trp Phe Leu Gly 275 280 285 Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe Ala Asn Glu Ser Gly 290 295 300 Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys Ala Arg Gln Val Phe 305 310 315 320 Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly 325 330 335 Ser Glu Val Asp Tyr Ala Gln Val Asn Asp Gln Val Thr Phe Ile Asp 340 345 350 Asn His Asp Met Glu Arg Phe His Thr Ser Asn Gly Asp Arg Arg Lys 355 360 365 Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala 370 375 380 Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly Gly Asn Asp Pro Asp 385 390 395 400 Asn Arg Ala Arg Leu Pro Ser Phe Ser Thr Thr Thr Ala Tyr Gln 405 410 415Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile Ala 420 425 430 Tyr Gly Ser Thr His Glu Arg Trp Ile Asn Asn Asp Val Ile Ile Tyr 435 440 445 Glu Arg Lys Phe Gly Asn Asn Val Ala Val Ala Ile Asn Arg Asn 450 460 Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val Thr Ser Leu Arg Arg 465 470 475 480 Ala Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu Asn Gly Asn Thr Leu 485 490 495 Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Pro Gly 500 510 Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala Thr Thr Pro Ile Ile 515 520 525 Gly Asn Val Gly Pro Met Met Ala Lys Pro Gly Val Thr Ile Thr Ile Page 24

535 540 530

Asp Gly Arg Gly Phe Gly Ser Gly Lys Gly Thr Val Tyr Phe Gly 545 555 Thr Ala Val Thr Gly Ala Asp Ile Val Ala Trp Glu Asp Thr Gln Ile 565 570 575 Gln Val Lys Ile Pro Ala Val Pro Gly Gly Ile Tyr Asp Ile Arg Val 580 585 590 Ala Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr Asp Asn Phe Glu Val 595 600 605 Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val Ile Asn Asn Ala Thr 610 615 620 Thr Ala Leu Gly Gln Asn Val Phe Leu Thr Gly Asn Val Ser Glu Leu 625 630 635 640 Gly Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro Met Tyr Asn Gln Val 645 650 655 Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly
660 665 670 Gln Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr 675 680 685 Trp Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr Pro Thr Ser Gly Thr 690 700

Ala Thr Val Asn Val Asn Trp Gln Pro 705 710

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PRT <212> <213> Bacillus sp. 38-2

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Leu Thr Leu Gly Leu Leu Ser Pro Val His Ala Ala Pro Asp Thr Ser 20 25 30

Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Phe 35 40 45

Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn Asn Pro Thr Gly Ala 50 60 Page 25

Ala Phe Asp Gly Ser Cys Thr Asn Leu Arg Leu Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met 85 90 95 Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Ile Tyr Ser 100 105 110 Val Ile Asn Tyr Ser Gly Val His Asn Thr Ala Tyr His Gly Tyr Trp 115 120 125 Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr Gly Thr Met Gln Asp 130 140 Phe Lys Asn Leu Ile Asp Thr Ala His Ala His Asn Ile Lys Val Ile 145 150 155 160 Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Ser Asp Asp Pro 165 170 175 Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn Gly Asn Leu Leu Gly 180 185 Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His His Tyr Gly Gly Thr 195 200 205 Asp Phe Ser Thr Ile Glu Asn Gly Ile Tyr Lys Asn Leu Tyr Asp Leu 210 215 220 Ala Asp Leu Asn His Asn Asn Ser Ser Val Asp Val Tyr Leu Lys Asp 225 230 235 240 Ala Ile Lys Met Trp Leu Asp Leu Gly Val Asp Gly Ile Arg Val Asp 245 250 255 Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Met Ser Thr 260 265 270 Ile Asn Asn Tyr Lys Pro Val Phe Asn Phe Gly Glu Trp Phe Leu Gly 275 280 285 Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe Ala Asn Glu Ser Gly 290 295 300 Met Ser Leu Leu Asp Phe Pro Phe Ala Gln Lys Ala Arg Gln Val Phe 305 310 315 320 Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly 325 330 335 Page 26

Ser Glu Val Asp Tyr Ala Gln Val Asp Gln Val Thr Phe Ile Asp 340 345 350 Asn His Asp Met Glu Arg Phe His Thr Ser Asn Gly Asp Arg Arg Lys 355 360 365 Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala 370 375 380 Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly Gly Asn Asp Pro Asp 385 390 395 400 Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Thr Thr Thr Ala Tyr Gln 405 410 415 Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile Ala 420 425 430 Tyr Gly Ser Thr Gln Glu Arg Trp Ile Asn Asn Asp Val Ile Ile Tyr 435 440 445 Glu Arg Lys Phe Gly Asn Asn Val Ala Val Ala Ile Asn Arg Asn 450 460 Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val Thr Ser Leu Pro Gln 465 470 475 480 Gly Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu Asn Gly Asn Thr Leu
485 490 495 Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Pro Gly 500 510 Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala Thr Ala Pro Ile Asn 515 520 525 Gly Asn Val Gly Pro Met Met Ala Lys Ala Gly Val Thr Ile Thr Ile 530 540 Asp Gly Arg Ala Ser Ala Arg Gln Gly Thr Val Tyr Phe Gly Thr Thr 545 550 555 560 Ala Val Thr Gly Ala Asp Ile Val Ala Trp Glu Asp Thr Gln Ile Gln 565 570 575 Val Lys Ile Leu Arg Val Pro Gly Gly Ile Tyr Asp Ile Arg Val Ala 580 585 Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr Asp Asn Phe Glu Val Leu 595 600 605 Page 27

Thr Gly Asp Gln Val Thr Val Arg Phe Val Ile Asn Asn Ala Thr Thr 610 620 Ala Leu Gly Gln Asn Val Phe Leu Thr Gly Asn Val Ser Glu Leu Gly 625 630 635 640 Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro Met Tyr Asn Gln Val Val 645 650 655 Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly Gln 660 670

Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr Trp 675 680 685

Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr Pro Thr Ser Gly Thr Ala 690 695 700

Thr Val Asn Val Asn Trp Gln Pro 705 710

<400> 11

Met Lys Lys Phe Leu Lys Ser Thr Ala Ala Leu Ala Leu Gly Leu Ser 10 15

Leu Thr Phe Gly Leu Phe Ser Pro Ala Gln Ala Ala Pro Asp Thr Ser 20 25 30

Val Ser Asn Lys Gln Asn Phe Ser Thr Asp Val Ile Tyr Gln Ile Phe 35 40 45

Thr Asp Arg Phe Ser Asp Gly Asn Pro Ala Asn Asn Pro Thr Gly Ala 50 60

Ala Phe Asp Gly Thr Cys Thr Asn Leu Arg Leu Tyr Cys Gly Gly Asp 65 70 75 80

Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Gly Met 85 90 95

Gly Val Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Ile Tyr Ser 100 105 110

Ile Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala Tyr His Gly Tyr Trp 115 120 125

<210> 11 713

<211>

<212> PRT

Bacillus circulans

Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr Gly Thr Ile Ala Asp 130 135 140 Phe Gln Asn Leu Ile Ala Ala Ala His Ala Lys Asn Ile Lys Val Ile 145 150 155 160 Tle Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Ser Asp Gln Pro
165 170 175 Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn Gly Thr Leu Leu Gly 180 185 190 Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His His Asn Gly Gly Thr 195 200 205 Asp Phe Ser Thr Thr Glu Asn Gly Ile Tyr Lys Asn Leu Tyr Asp Leu 210 215 220 Ala Asp Leu Asn His Asn Asn Ser Thr Val Asp Val Tyr Leu Lys Asp 225 230 235 Ala Ile Lys Met Trp Leu Asp Leu Gly Ile Asp Gly Ile Arg Met Asp 245 250 255 Ala Val Lys His Met Pro Phe Gly Trp Gln Lys Ser Phe Met Ala Ala 260 265 270 Val Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly Glu Trp Phe Leu Gly 275 280 285 Val Asn Glu Val Ser Pro Glu Asn His Lys Phe Ala Asn Glu Ser Gly 290 295 300 Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys Val Arg Gln Val Phe 305 310 315 320 Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys Ala Met Leu Glu Gly 325 330 335 Ser Ala Ala Asp Tyr Ala Gln Val Asp Asp Gln Val Thr Phe Ile Asp 340 345 350 Asn His Asp Met Glu Arg Phe His Ala Ser Asn Ala Asn Arg Arg Lys 355 360 365 Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala 370 375 380 Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Ser Gly Gly Thr Asp Pro Asp 385 390 395 400

Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Ser Thr Thr Ala Tyr Gln 405 410 415 Val Ile Gln Lys Leu Ala Pro Leu Arg Lys Cys Asn Pro Ala Ile Ala 420 425 430 Tyr Gly Ser Thr Gln Glu Arg Trp Ile Asn Asn Asp Val Leu Ile Tyr 435 440 445 Glu Arg Lys Phe Gly Ser Asn Val Ala Val Ala Val Asn Arg Asn 450 455 460 Leu Asn Ala Pro Ala Ser Ile Ser Gly Leu Val Thr Ser Leu Pro Gln 465 470 475 480 Gly Ser Tyr Asn Asp Val Leu Gly Gly Leu Leu Asn Gly Asn Thr Leu
485 490 495 Ser Val Gly Ser Gly Gly Ala Ala Ser Asn Phe Thr Leu Ala Ala Gly 500 505 Gly Thr Ala Val Trp Gln Tyr Thr Ala Ala Thr Ala Thr Pro Thr Ile 515 520 525 Gly His Val Gly Pro Met Met Ala Lys Pro Gly Val Thr Ile Thr Ile 530 540 Asp Gly Arg Gly Phe Gly Ser Ser Lys Gly Thr Val Tyr Phe Gly Thr 545 550 555 Thr Ala Val Ser Gly Ala Asp Ile Thr Ser Trp Glu Asp Thr Gln Ile 565 570 575 Lys Val Lys Ile Pro Ala Val Ala Gly Gly Asn Tyr Asn Ile Lys Val 580 585 590 Ala Asn Ala Ala Gly Thr Ala Ser Asn Val Tyr Asp Asn Phe Glu Val 595 600 605 Leu Ser Gly Asp Gln Val Ser Val Arg Phe Val Val Asn Asn Ala Thr 610 615 620 Thr Ala Leu Gly Gln Asn Val Tyr Leu Thr Gly Ser Val Ser Glu Leu 625 630 635 640 Gly Asn Trp Asp Pro Ala Lys Ala Ile Gly Pro Met Tyr Asn Gln Val 645 650 655 Val Tyr Gln Tyr Pro Asn Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly
660 665 670

Lys Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln Gly Ser Thr Val Thr 675 680 685

Trp Glu Gly Gly Ser Asn His Thr Phe Thr Ala Pro Ser Ser Gly Thr 690 695 700

Ala Thr Ile Asn Val Asn Trp Gln Pro 705 710

<210> <211> 12 686

PRT Bacillus sp.

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Asn Pro Thr Gly Ala Ala Phe Asp Gly Ser Cys Thr Asn Leu Arg Leu 35 40 45

Tyr Cys Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly 50 60

Tyr Leu Thr Gly Met Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val 65 70 75 80

Glu Asn Ile Tyr Ser Val Ile Asn Tyr Ser Gly Val Asn Asn Thr Ala 85 90 95

Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro Ala Tyr 100 105 110

Gly Thr Met Gln Asp Phe Lys Asn Leu Ile Asp Thr Ala His Ala His 115 120 125

Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala 130 135 140

Ser Ser Asp Asp Pro Ser Phe Ala Glu Asn Gly Arg Leu Tyr Asp Asn 145 150 155 160

Gly Asn Leu Leu Gly Gly Tyr Thr Asn Asp Thr Gln Asn Leu Phe His 165 170 175

His Tyr Gly Gly Thr Asp Phe Ser Thr Ile Glu Asn Gly Ile Tyr Lys 180 185 190

Asn Leu Tyr Asp Leu Ala Asp Leu Asn His Asn Asn Ser Ser Val Asp 195 200 205 Val Tyr Leu Lys Asp Ala Ile Lys Met Trp Leu Asp Leu Gly Val Asp 210 220 Gly Ile Arg Val Asp Ala Val Lys His Met Pro Phe Gly Trp Gln Lys 225 230 235 240 Ser Phe Met Ser Thr Ile Asn Asn Tyr Lys Pro Val Phe Thr Phe Gly 245 250 255 Glu Trp Phe Leu Gly Val Asn Glu Ile Ser Pro Glu Tyr His Gln Phe 260 265 270 Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys 275 280 285 Ala Arg Gln Val Phe Arg Asp Asn Thr Asp Asn Met Tyr Gly Leu Lys 290 300 Ala Met Leu Glu Gly Ser Glu Val Asp Tyr Ala Gln Val Asn Asp Gln 305 310 315 320 Val Thr Phe Ile Asp Asn His Asp Met Glu Arg Phe His Thr Ser Asn 325 330 335 Gly Asp Arg Arg Lys Leu Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser 340 345 350 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Ser Glu Gln Tyr Met Ser Gly 355 360 365 Gly Asn Asp Pro Asp Asn Arg Ala Arg Ile Pro Ser Phe Ser Thr Thr 370 380 Thr Thr Ala Tyr Gln Val Ile Gln Lys Leu Ala Pro Leu Arg Lys 385 390 395 Asn Pro Ala Ile Ala Tyr Gly Ser Thr Glu Arg Trp Ile Asn Asn 405 410 415Asp Val Ile Ile Tyr Glu Arg Lys Phe Gly Asn Asn Val Ala Val Val 420 425 430 Ala Ile Asn Arg Asn Met Asn Thr Pro Ala Ser Ile Thr Gly Leu Val 435 440 445 Thr Ser Leu Pro Gln Gly Ser Tyr Asn Asp Val Leu Gly Gly Ile Leu 450 460

Asn Gly Asn Thr Leu Thr Val Gly Ala Gly Gly Ala Ala Ser Asn Phe 465 470 480 Thr Leu Ala Pro Gly Gly Thr Ala Val Trp Gln Tyr Thr Thr Asp Ala 485 490 495 Thr Ala Pro Ile Ile Gly Asn Val Gly Pro Met Met Ala Lys Pro Gly 500 505 Val Thr Ile Asp Gly Arg Gly Phe Gly Ser Gly Lys Gly Thr 515 525 Val Tyr Phe Gly Thr Thr Ala Val Thr Gly Ala Asp Ile Val Ala Trp 530 540 Glu Asp Thr Gln Ile Gln Val Lys Ile Pro Ala Val Pro Gly Gly Ile 545 550 555 560 Tyr Asp Ile Arg Val Ala Asn Ala Ala Gly Ala Ala Ser Asn Ile Tyr 565 570 575 Asp Asn Phe Glu Val Leu Thr Gly Asp Gln Val Thr Val Arg Phe Val 580 585 590 Ile Asn Asn Ala Thr Thr Ala Leu Gly Gln Asn Val Phe Leu Thr Gly 595 600 605 Asn Val Ser Glu Leu Gly Asn Trp Asp Pro Asn Asn Ala Ile Gly Pro 610 615 620 Met Tyr Asn Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val 625 630 635 640 Ser Val Pro Ala Gly Gln Thr Ile Glu Phe Lys Phe Leu Lys Lys Gln 645 650 655 Gly Ser Thr Val Thr Trp Glu Gly Gly Ala Asn Arg Thr Phe Thr Thr 660 665 670 Pro Thr Ser Gly Thr Ala Thr Met Asn Val Asn Trp Gln Pro 675 680 685

<210> 13

<211> 704

<212> PRT

<213> Bacillus ohbensis

<400> 13

Met Lys Asn Leu Thr Val Leu Leu Lys Thr Ile Pro Leu Ala Leu Leu 1 5 10 15

Leu Phe Ile Leu Leu Ser Leu Pro Thr Ala Ala Gln Ala Asp Val Thr Page 33

20

30

25 ⁻

Asn Lys Val Asn Tyr Thr Arg Asp Val Ile Tyr Gln Ile Val Thr Asp 40 45 Arg Phe Ser Asp Gly Asp Pro Ser Asn Asn Pro Thr Gly Ala Ile Tyr 50 55 60 Ser Gln Asp Cys Ser Asp Leu His Lys Tyr Cys Gly Gly Asp Trp Gln 70 75 80 Gly Ile Ile Asp Lys Ile Asn Asp Gly Tyr Leu Thr Asp Leu Gly Ile 85 90 95 Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Val Tyr Ala Leu His $100 \hspace{1cm} 105 \hspace{1cm} 110$ Pro Ser Gly Tyr Thr Ser Tyr His Gly Tyr Trp Ala Arg Asp Tyr Lys 115 120 125 Arg Thr Asn Pro Phe Tyr Gly Asp Phe Ser Asp Phe Asp Arg Leu Met 130 140 Asp Thr Ala His Ser Asn Gly Ile Lys Val Ile Met Asp Phe Thr Pro 145 150 155 160 Asn His Ser Ser Pro Ala Leu Glu Thr Asp Pro Ser Tyr Ala Glu Asn 165 170 175 Gly Ala Val Tyr Asn Asp Gly Val Leu Ile Gly Asn Tyr Ser Asn Asp 180 185 190Pro Asn Asn Leu Phe His His Asn Gly Gly Thr Asp Phe Ser Ser Tyr 195 200 205 Glu Asp Ser Ile Tyr Arg Asn Leu Tyr Asp Leu Ala Asp Tyr Asp Leu 210 220 Asn Asn Thr Val Met Asp Gln Tyr Leu Lys Glu Ser Ile Lys Leu Trp 225 230 240 Leu Asp Lys Gly Ile Asp Gly Ile Arg Val Asp Ala Val Lys His Met 245 250 255 Ser Glu Gly Trp Gln Thr Ser Leu Met Ser Asp Ile Tyr Ala His Glu 260 265 270 Pro Val Phe Thr Phe Gly Glu Trp Phe Leu Gly Ser Gly Glu Val Asp 275 280 285 Pro Gln Asn His His Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Page 34

290 295 300

Phe Gln Phe Gly Gln Thr Ile Arg Asp Val Leu Met Asp Gly Ser 315 Asn Trp Tyr Asp Phe Asn Glu Met Ile Ala Ser Thr Glu Glu Asp Tyr 325 330 335 Asp Glu Val Ile Asp Gln Val Thr Phe Ile Asp Asn His Asp Met Ser 340 345 Arg Phe Ser Phe Glu Gln Ser Ser Asn Arg His Thr Asp Ile Ala Leu 355 360 Ala Val Leu Leu Thr Ser Arg Gly Val Pro Thr Ile Tyr Tyr Gly Thr 370 375 380 Glu Gln Tyr Leu Thr Gly Gly Asn Asp Pro Glu Asn Arg Lys Pro Met 385 390 395 400 Ser Asp Phe Asp Arg Thr Thr Asn Ser Tyr Gln Ile Ile Ser Thr Leu 405 410 415Ala Ser Leu Arg Gln Asn Asn Pro Ala Leu Gly Tyr Gly Asn Thr Ser 420 425 430 Glu Arg Trp Ile Asn Ser Asp Val Tyr Ile Tyr Glu Arg Ser Phe Gly
435
440 Asp Ser Val Val Leu Thr Ala Val Asn Ser Gly Asp Thr Ser Tyr Thr 450 460 Ile Asn Asn Leu Asn Thr Ser Leu Pro Gln Gly Gln Tyr Thr Asp Glu 465 470 475 480 Leu Gln Gln Leu Leu Asp Gly Asn Glu Ile Thr Val Asn Ser Asn Gly
485 490 495 Ala Val Asp Ser Phe Gln Leu Ser Ala Asn Gly Val Ser Val Trp Gln 500 510 Ile Thr Glu Glu His Ala Ser Pro Leu Ile Gly His Val Gly Pro Met 515 520 525 Met Gly Lys His Gly Asn Thr Val Thr Ile Thr Gly Glu Gly Phe Gly 530 540 Asp Asn Glu Gly Ser Val Leu Phe Asp Ser Asp Phe Ser Asp Val Leu 545 550 555 Ser Trp Ser Asp Thr Lys Ile Glu Val Ser Val Pro Asp Val Thr Ala Page 35

> 565 570 575

Gly His Tyr Asp Ile Ser Val Val Asn Ala Gly Asp Ser Gln Ser Pro 580 585 Thr Tyr Asp Lys Phe Glu Val Leu Thr Gly Asp Gln Val Ser Ile Arg 595 600 605 Phe Ala Val Asn Asn Ala Thr Thr Ser Leu Gly Thr Asn Leu Tyr Met Val Gly Asn Val Asn Glu Leu Gly Asn Trp Asp Pro Asp Gln Ala Ile 625 630 635 640 Gly Pro Met Phe Asn Gln Val Met Tyr Gln Tyr Pro Thr Trp Tyr Tyr 645 650 655 Asp Ile Ser Val Pro Ala Glu Glu Asn Leu Glu Tyr Lys Phe Ile Lys 660 665 670 Lys Asp Ser Ser Gly Asn Val Val Trp Glu Ser Gly Asn Asn His Thr 675 680 685

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Met Asn Asp Leu Asn Asp Phe Leu Lys Thr Ile Leu Leu Ser Phe Ile 10 15

Tyr Thr Thr Pro Ala Thr Gly Thr Asp Thr Val Leu Val Asp Trp Gln 690 695 700

Phe Phe Leu Leu Ser Leu Pro Thr Val Ala Glu Ala Asp Val Thr 20 25 30

Asn Lys Val Asn Tyr Ser Lys Asp Val Ile Tyr Gln Ile Val Thr Asp 40 45

Arg Phe Ser Asp Gly Asn Pro Gly Asn Asn Pro Ser Gly Ala Ile Phe 50 60

Ser Gln Asn Cys Ile Asp Leu His Lys Tyr Cys Gly Gly Asp Trp Gln 65 70 75 80

Gly Ile Ile Asp Lys Ile Asn Asp Gly Tyr Leu Thr Asp Leu Gly Ile

Thr Ala Leu Trp Ile Ser Gln Pro Val Glu Asn Val Tyr Ala Leu His 100 105 110 Page 36

<210> <211> 14 703

Bacillus sp. 1-1

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Glu Gln Tyr Val Thr Gly Gly Asn Asp Pro Glu Asn Arg Lys Pro Leu 385 390 395 400 Lys Thr Phe Asp Arg Ser Thr Asn Ser Tyr Gln Ile Ile Ser Lys Leu 405 415 Ala Ser Leu Arg Gln Thr Asn Ser Ala Leu Gly Tyr Gly Thr Thr 420 425 430 Glu Arg Trp Leu Asn Glu Asp Ile Tyr Ile Tyr Glu Arg Thr Phe Gly 445 Asn Ser Ile Val Leu Thr Ala Val Asn Ser Ser Asn Ser Asn Gln Thr 450 455 460 Ile Thr Asn Leu Asn Thr Ser Leu Pro Gln Gly Asn Tyr Thr Asp Glu 465 470 475 480 Leu Gln Gln Arg Leu Asp Gly Asn Thr Ile Thr Val Asn Ala Asn Gly 485 490 495 Ala Val Asn Ser Phe Gln Leu Arg Ala Asn Ser Val Ala Val Trp Gln 500 505 510 Val Ser Asn Pro Ser Thr Ser Pro Leu Ile Gly Gln Val Gly Pro Met 515 520 525 Met Gly Lys Ala Gly Asn Thr Ile Thr Val Ser Gly Glu Gly Phe Gly 530 540 Asp Glu Arg Gly Ser Val Leu Phe Asp Ser Thr Ser Ser Glu Ile Ile 545 550 555 Ser Trp Ser Asn Thr Lys Ile Ser Val Lys Val Pro Asn Val Ala Gly 565 570 Gly Tyr Tyr Asp Leu Ser Val Val Thr Ala Ala Asn Ile Lys Ser Pro 580 585 590 Thr Tyr Lys Glu Phe Glu Val Leu Ser Gly Asn Gln Val Ser Val Arg 595 600 605 Phe Gly Val Asn Asn Ala Thr Thr Ser Pro Gly Thr Asn Leu Tyr Ile 610 620 Val Gly Asn Val Asn Glu Leu Gly Asn Trp Asp Ala Asp Lys Ala Ile 625 630 635 640 Gly Pro Met Phe Asn Gln Val Met Tyr Gln Tyr Pro Thr Trp Tyr Tyr 645 650 655 Page 38

Asp Ile Ser Val Pro Ala Gly Lys Asn Leu Glu Tyr Lys Tyr Ile Lys 660 665 670

Lys Asp Gln Asn Gly Asn Val Val Trp Gln Ser Gly Asn Asn Arg Thr 675 680 685

Tyr Thr Ser Pro Thr Thr Gly Thr Asp Thr Val Met Ile Asn Trp 690 695 700

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Ala Ile Phe Ile Val Ser Asp Thr Gln Lys Val Thr Val Glu Ala Ala 20 25 30

Gly Asn Leu Asn Lys Val Asn Phe Thr Ser Asp Val Val Tyr Gln Ile 35 40 45

Val Val Asp Arg Phe Val Asp Gly Asn Thr Ser Asn Asn Pro Ser Gly 50 60

Ala Leu Phe Ser Ser Gly Cys Thr Asn Leu Arg Lys Tyr Cys Gly Gly 65 70 75 80

Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly Tyr Leu Thr Asp 85 90 95

Met Gly Val Thr Ala Ile Trp Ile Ser Gln Pro Val Glu Asn Val Phe 100 105 110

Ser Val Met Asn Asp Ala Ser Gly Ser Ala Ser Tyr His Gly Tyr Trp 115 120 125

Ala Arg Asp Phe Lys Lys Pro Asn Pro Phe Phe Gly Thr Leu Ser Asp 130 140

Phe Gln Arg Leu Val Asp Ala Ala His Ala Lys Gly Ile Lys Val Ile 145 150 155 160

Ile Asp Phe Ala Pro Asn His Thr Ser Pro Ala Ser Glu Thr Asn Pro 165 170 175

Ser Tyr Met Glu Asn Gly Arg Leu Tyr Asp Asn Gly Thr Leu Leu Gly 180 185 190

<210> 15 <211> 711

<212> PRT

<213> Bacillus stearothermophilus

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Ser Ser Ser Asn Tyr Ser Ile Thr Gly Leu Phe Thr Ala Leu Pro Ala 465 470 475 480 Gly Thr Tyr Thr Asp Gln Leu Gly Gly Leu Leu Asp Gly Asn Thr Ile 485 490 495 Gln Val Gly Ser Asn Gly Ser Val Asn Ala Phe Asp Leu Gly Pro Gly 500 505 510 Glu Val Gly Val Trp Ala Tyr Ser Ala Thr Glu Ser Thr Pro Ile Ile 515 520 525 Gly His Val Gly Pro Met Met Gly Gln Val Gly His Gln Val Thr Ile 530 540 Asp Gly Glu Gly Phe Gly Thr Asn Thr Gly Thr Val Lys Phe Gly Thr 545 550 555 Thr Ala Ala Asn Val Val Ser Trp Ser Asn Asn Gln Ile Val Val Ala 565 570 575 Val Pro Asn Val Ser Pro Gly Lys Tyr Asn Ile Thr Val Gln Ser Ser 580 585 590 Ser Gly Gln Thr Ser Ala Ala Tyr Asp Asn Phe Glu Val Leu Thr Asn 595 600 605 Asp Gln Val Ser Val Arg Phe Val Val Asn Asn Ala Thr Thr Asn Leu 610 615 620 Gly Gln Asn Ile Tyr Ile Val Gly Asn Val Tyr Glu Leu Gly Asn Trp 625 630 635 640 Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn Gln Val Val Tyr Ser 645 650 655 Tyr Pro Thr Trp Tyr Ile Asp Val Ser Val Pro Glu Gly Lys Thr Ile 660 665 670 Glu Phe Lys Phe Ile Lys Lys Asp Ser Gln Gly Asn Val Thr Trp Glu 675 680 685 Ser Gly Ser Asn His Val Tyr Thr Thr Pro Thr Asn Thr Thr Gly Lys 690 695 700 Ile Ile Val Asp Trp Gln Asn 705 710

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Ile Ala Leu Asn Thr Phe Phe Cys Ser Met Gln Thr Ile Ala Ala Glu $20 \hspace{1cm} 25 \hspace{1cm} 30$

Pro Glu Glu Thr Tyr Leu Asp Phe Arg Lys Glu Thr Ile Tyr Phe Leu 35 40 45

Phe Leu Asp Arg Phe Ser Asp Gly Asp Pro Ser Asn Asn Ala Gly Phe $50 \hspace{1.5cm} 60$

Asn Ser Ala Thr Tyr Asp Pro Asn Asn Leu Lys Lys Tyr Thr Gly Gly 65 70 75 80

Asp Leu Arg Gly Leu Ile Asn Lys Leu Pro Tyr Leu Lys Ser Leu Gly $85 \hspace{1cm} 90 \hspace{1cm} 95$

Val Thr Ser Ile Trp Ile Thr Pro Pro Ile Asp Asn Val Asn Asn Thr 100 105 110

Asp Ala Ala Gly Asn Thr Gly Tyr His Gly Tyr Trp Gly Arg Asp Tyr 115 120 125

Phe Arg Ile Asp Glu His Phe Gly Asn Leu Asp Asp Phe Lys Glu Leu 130 140

Thr Ser Leu Met His Ser Pro Asp Tyr Asn Met Lys Leu Val Leu Asp 145 150 155 160

Tyr Ala Pro Asn His Ser Asn Ala Asn Asp Glu Asn Glu Phe Gly Ala 165 170 175

Leu Tyr Arg Asp Gly Val Phe Ile Thr Asp Tyr Pro Thr Asn Val Ala 180 185 190

Ala Asn Thr Gly Trp Tyr His His Asn Gly Gly Val Thr Asn Trp Asn 195 200 205

Asp Phe Phe Gln Val Lys Asn His Asn Leu Phe Asn Leu Ser Asp Leu 210 215 220

Asn Gln Ser Asn Thr Asp Val Tyr Gln Tyr Leu Leu Asp Gly Ser Lys 225 230 235 240

Phe Trp Ile Asp Ala Gly Val Asp Ala Ile Arg Ile Asp Ala Ile Lys 245 250 255

His Met Asp Lys Ser Phe Ile Gln Lys Trp Thr Ser Asp Ile Tyr Asp 260 265 270 Tyr Ser Lys Ser Ile Gly Arg Glu Gly Phe Phe Phe Gly Glu Trp 275 280 285 Phe Gly Ala Ser Ala Asn Thr Thr Gly Val Asp Gly Asn Ala Ile 290 295 300 Asp Tyr Ala Asn Thr Ser Gly Ser Ala Leu Leu Asp Phe Gly Phe Arg 305 310 315 320 Asp Thr Leu Glu Arg Val Leu Val Gly Arg Ser Gly Asn Thr Met Lys 325 330 335 Thr Leu Asn Ser Tyr Leu Ile Lys Arg Gln Thr Val Phe Thr Ser Asp 340 345 Asp Trp Gln Val Val Phe Met Asp Asn His Asp Met Ala Arg Ile Gly 355 360 Thr Ala Leu Arg Ser Asn Ala Thr Thr Phe Gly Pro Gly Asn Asn Glu 370 380 Thr Gly Gly Ser Gln Ser Glu Ala Phe Ala Gln Lys Arg Ile Asp Leu 385 390 395 400 Gly Leu Val Ala Thr Met Thr Val Arg Gly Ile Pro Ala Ile Tyr Tyr 405 410 415 Gly Thr Glu His Tyr Ala Ala Asn Phe Thr Ser Asn Ser Phe Gly Gln
420 425 430 Val Gly Ser Asp Pro Tyr Asn Arg Glu Lys Met Pro Gly Phe Asp Thr 445 445 Glu Ser Glu Ala Phe Ser Ile Ile Lys Thr Leu Gly Asp Leu Arg Lys 450 460 Ser Ser Pro Ala Ile Gln Asn Gly Thr Tyr Thr Glu Leu Trp Val Asn 465 470 475 480 Asp Asp Ile Leu Val Phe Glu Arg Arg Ser Gly Asn Asp Ile Val Ile 485 490 495 Val Ala Leu Asn Arg Gly Glu Ala Asn Thr Ile Asn Val Lys Asn Ile 500 505 510 Ala Val Pro Asn Gly Val Tyr Pro Ser Leu Ile Gly Asn Asn Ser Val 515 520 525

Ser Val Ala Asn Lys Arg Thr Thr Leu Thr Leu Met Gln Asn Glu Ala 530 535 540

Val Val Ile Arg Ser Gln Ser Asp Asp Ala Glu Asn Pro Thr Val Gln 545 550 560

Ser Ile Asn Phe Thr Cys Asn Asn Gly Tyr Thr Ile Ser Gly Gln Ser 565 570 575

Val Tyr Ile Ile Gly Asn Ile Pro Gln Leu Gly Gly Trp Asp Leu Thr 580 585

Lys Ala Val Lys Ile Ser Pro Thr Gln Tyr Pro Gln Trp Ser Ala Ser 595 600 605

Leu Glu Leu Pro Ser Asp Leu Asn Val Glu Trp Lys Cys Val Lys Arg 610 615 620

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Asp Arg Phe Tyr Asp Gly Asp Thr Thr Asn Asn Asn Pro Ala Lys Ser 20 25 30

Tyr Gly Leu Tyr Asp Pro Thr Lys Ser Lys Trp Lys Met Tyr Trp Gly 35 40 45

Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln Leu 50 60

Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp Thr 65 70 75 80

Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg Asp $85 \hspace{1.5cm} 90 \hspace{1.5cm} 95$

Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp Thr 100 105 110

Leu Val Asn Asp Ala His Gln Asn Gly Ile Lys Val Ile Val Asp Phe Page 44

<210> 17

<211> 686 <212> PRT

<213> Bacillus stearothermophilus

115 120 125

Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe Ala 130 140 Glu Gly Gly Ala Leu Tyr Asn Asn Gly Thr Tyr Met Gly Asn Tyr Phe 145 150 155 160 Asp Asp Ala Thr Lys Gly Tyr Phe His His Asn Gly Asp Ile Ser Asn 165 170 175 Trp Asp Asp Arg Tyr Glu Ala Gln Trp Lys Asn Phe Thr Asp Pro Ala 180 185 190 Gly Phe Ser Leu Ala Asp Leu Ser Gln Glu Asn Gly Thr Ile Ala Gln 195 200 205 Tyr Leu Thr Asp Ala Ala Val Gln Leu Val Ala His Gly Ala Asp Gly 210 220 Leu Arg Ile Asp Ala Val Lys His Phe Asn Ser Gly Phe Ser Lys Ser 225 230 235 240 Leu Ala Asp Lys Leu Tyr Gln Lys Lys Asp Ile Phe Leu Val Gly Glu 245 250 255 Trp Tyr Gly Asp Asp Pro Gly Thr Ala Asn His Leu Glu Lys Val Arg 260 265 270 Tyr Ala Asn Asn Ser Gly Val Asn Val Leu Asp Phe Asp Leu Asn Thr 275 280 285 Ile Arg Asn Val Phe Gly Thr Phe Thr Gln Thr Met Tyr Asp Leu 290 300Asn Asn Met Val Asn Gln Thr Gly Asn Glu Tyr Lys Tyr Lys Glu Asn 305 310 315 320 Leu Ile Thr Phe Ile Asp Asn His Asp Met Ser Arg Phe Leu Ser Val Asn Ser Asn Lys Ala Asn Leu His Gln Ala Leu Ala Phe Ile Leu Thr 340 345 350 Ser Arg Gly Thr Pro Ser Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Ala 355 360 Gly Gly Asn Asp Pro Tyr Asn Arg Gly Met Met Pro Ala Phe Asp Thr 370 380 Thr Thr Ala Phe Lys Glu Val Ser Thr Leu Ala Gly Leu Arg Arg Page 45

385 390 395 400

Asn Asn Ala Ala Ile Gln Tyr Gly Thr Thr Thr Gln Arg Trp Ile Asn 405 410 415Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Phe Asn Asp Val Val Leu 420 425 430 Val Ala Ile Asn Arg Asn Thr Gln Ser Ser Tyr Ser Ile Ser Gly Leu 435 440 445 Gln Thr Ala Leu Pro Asn Gly Ser Tyr Ala Asp Tyr Leu Ser Gly Leu 450 460 Leu Gly Gly Asn Gly Ile Ser Val Ser Asn Gly Ser Val Ala Ser Phe 465 470 475 480 Thr Leu Ala Pro Gly Ala Val Ser Val Trp Gln Tyr Ser Thr Ser Ala 485 490 495 Ser Ala Pro Gln Ile Gly Ser Val Ala Pro Asn Met Gly Ile Pro Gly 500 505 510Asn Val Val Thr Ile Asp Gly Lys Gly Phe Gly Thr Thr Gln Gly Thr 515 520 525 Val Thr Phe Gly Gly Val Thr Ala Thr Val Lys Ser Trp Thr Ser Asn 530 540 Arg Ile Glu Val Tyr Val Pro Asn Met Ala Ala Gly Leu Thr Asp Val 545 550 560 Lys Val Thr Ala Gly Gly Val Ser Ser Asn Leu Tyr Ser Tyr Asn Ile 565 570 575 Leu Ser Gly Thr Gln Thr Ser Val Val Phe Thr Val Lys Ser Ala Pro 580 585 590 Pro Thr Asn Leu Gly Asp Lys Ile Tyr Leu Thr Gly Asn Ile Pro Glu 595 600 605 Leu Gly Asn Trp Ser Thr Asp Thr Ser Gly Ala Val Asn Asn Ala Gln 610 615 620 Gly Pro Leu Leu Ala Pro Asn Tyr Pro Asp Trp Phe Tyr Val Phe Ser 625 630 635 Val Pro Ala Gly Lys Thr Ile Gln Phe Lys Phe Phe Ile Lys Arg Ala 645 650 655 Asp Gly Thr Ile Gln Trp Glu Asn Gly Ser Asn His Val Ala Thr Thr

Page 46

660 665 670

Pro Thr Gly Ala Thr Gly Asn Ile Thr Val Thr Trp Gln Asn 675 680 685

<210> 18

<211> 683 <212> PRT

<213> Bacillus stearothermophilus

<400> 18

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Asp Arg Phe Tyr Asp Gly Asp Thr Thr Asn Asn Asn Pro Ala Lys Ser 20 25 30

Tyr Gly Leu Tyr Asp Pro Thr Lys Ser Lys Trp Lys Met Tyr Trp Gly 35 40 45

Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln Leu 50 60

Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp Thr 65 70 75 80

Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg Asp 85 90 95

Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp Thr 100 105 110

Leu Val Asn Asp Ala His Gln Asn Gly Ile Lys Val Ile Val Asp Phe 115 120 125

Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe Ala 130 135 140

Glu Gly Gly Ala Leu Tyr Asn Asn Gly Thr Tyr Met Gly Asn Tyr Phe 145 150 155 160

Asp Asp Ala Thr Lys Gly Tyr Phe His His Asn Gly Asp Ile Ser Asn 165 170 175

Trp Asp Asp Arg Ala Glu Ala Gln Trp Lys Asn Phe Thr Asp Pro Ala 180 185

Gly Phe Ser Leu Ala Asp Leu Ser Gln Glu Asn Gly Thr Ile Ala Gln 195 200 205

Tyr Leu Thr Asp Ala Ala Val Gln Leu Val Ala His Gly Ala Asp Gly 210 215 220

Leu Arg Ile Asp Ala Val Lys His Phe Asn Ser Gly Phe Ser Lys Ser 225 230 240 Leu Ala Asp Lys Leu Tyr Gln Lys Lys Asp Ile Phe Leu Val Gly Glu 245 250 255 Trp Tyr Gly Asp Asp Pro Gly Thr Ala Asn His Leu Glu Lys Val Arg 260 265 270 Tyr Ala Asn Asn Ser Gly Val Asn Val Leu Asp Phe Asp Leu Asn Thr 275 280 285 Val Ile Arg Asn Val Phe Gly Thr Phe Thr Gln Thr Met Tyr Asp Leu 290 295 300 Asn Asn Met Val Asn Gln Thr Gly Asn Glu Tyr Lys Tyr Lys Glu Asn 305 310 315 320 Leu Ile Thr Phe Ile Asp Asn His Asp Met Ser Arg Phe Leu Ser Val Asn Ser Asn Lys Ala Asn Leu His Gln Ala Leu Ala Phe Ile Leu Thr 340 345 350 Ser Arg Gly Thr Pro Ser Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Ala 355 360 365 Gly Gly Asn Asp Pro Tyr Asn Arg Gly Met Met Pro Ala Phe Asp Thr 370 380 Thr Thr Ala Phe Lys Glu Val Ser Thr Leu Ala Gly Leu Arg Arg 385 390 400 Asn Asn Ala Ala Ile Gln Tyr Gly Thr Thr Gln Arg Trp Ile Asn 405 410 415 Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Phe Asn Asp Val Val Leu 420 425 Val Ala Ile Asn Arg Asn Thr Gln Ser Ser Tyr Ser Ile Ser Gly Leu 435 440 445 Gln Thr Ala Leu Pro Asn Gly Ser Tyr Ala Asp Tyr Leu Ser Gly Leu 450 460 Leu Gly Gly Asn Gly Ile Ser Val Ser Asn Gly Ser Val Ala Ser Phe 465 470 475 480 Thr Leu Ala Pro Gly Ala Val Ser Val Trp Gln Tyr Ser Thr Thr Thr 485 490 495 Page 48

Asn Pro Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly 500 510 Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly Gln 515 520 525 Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp Thr 530 540 Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn Ile 545 550 560 Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn Ile 565 570 575 Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn Asn 580 585 590 Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala 595 600 605 Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn 610 615 620 Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro 625 630 635 640 Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser Thr 645 650 655 val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr Ser 660 665 670 Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro 675 680

Ala Pro Asp Thr Ser Val Ser Asn Val Val Asn Tyr Ser Thr Asp Val 10 15

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Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys 35 40 45

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Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Thr Gly 325 330 335 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser 340 345 350 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly 355 360 365 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asp Thr Thr 370 380 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser 385 390 395 400 Asn Pro Ala Ile Ala Tyr Gly Thr Gln Lys Gln Arg Trp Ile Asn Asn 405 410 415 Asp Val Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu Val 420 425 430 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu Tyr 435 440 445 Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu Leu 450 460 Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro Phe 465 470 475 480 Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Ser Ala 485 490 495 Ser Ala Pro Gln Ile Gly Ser Val Ala Pro Asn Met Gly Ile Pro Gly 500 505 510Asn Val Val Thr Ile Asp Gly Lys Gly Phe Gly Thr Thr Gln Gly Thr 515 520 525 Val Thr Phe Gly Gly Val Thr Ala Thr Val Lys Ser Trp Thr Ser Asn 530 540 Ser Ile Glu Val Tyr Val Pro Asn Met Ala Ala Gly Leu Thr Asp Val 545 550 555 560 Lys Val Thr Ala Gly Gly Val Ser Ser Asn Leu Tyr Ser Tyr Asn Ile 565 570 575 Leu Ser Gly Thr Gln Thr Ser Val Val Phe Thr Val Lys Ser Ala Pro 580 585 590

Pro Thr Asn Leu Gly Asp Lys Ile Tyr Leu Thr Gly Asn Ile Pro Glu 595 600 605 Leu Gly Asn Trp Ser Thr Asp Thr Ser Gly Ala Val Asn Asn Ala Gln 610 615 Gly Pro Leu Leu Ala Pro Asn Tyr Pro Asp Trp Phe Tyr Val Phe Ser 625 630 640 Val Pro Ala Gly Lys Thr Ile Gln Phe Lys Phe Phe Ile Lys Arg Ala 645 650 655 Asp Gly Thr Ile Gln Trp Glu Asn Gly Ser Asn His Val Ala Thr Thr 660 665 670 Pro Thr Gly Ala Thr Gly Asn Ile Thr Val Thr Trp Gln Asn 675 680 685

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Tyr Gly Leu Tyr Asp Pro Thr Lys Ser Lys Trp Lys Met Tyr Trp Gly 35 40 45

Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln Leu 50 60

Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp Thr 65 70 75 80

Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg Asp 85 90 95

Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp Thr 100 105 110

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Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe Ala 130 135 140

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Bacillus stearothermophilus

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Asn Asp Val Tyr Ile Tyr Glu Arg Lys Phe Phe Asn Asp Val Val Leu 420 425 Val Ala Ile Asn Arg Asn Thr Gln Ser Ser Tyr Ser Ile Ser Gly Leu 435 440 445 Gln Thr Ala Leu Pro Asn Gly Ser Tyr Ala Asp Tyr Leu Ser Gly Leu 450 460 Leu Gly Gly Asn Gly Ile Ser Val Ser Asn Gly Ser Val Ala Ser Phe 465 470 475 480 Thr Leu Ala Pro Gly Ala Val Ser Val Trp Gln Tyr Ser Thr Ser Ala 485 490 495 Ser Ala Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly 500 510 Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly Gln 515 520 Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp Thr 530 535 540 Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn Ile 545 550 555 560 Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn Ile 565 570 575 Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn Asn 580 585 Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala 595 600 Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn 610 615 620 Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro 625 630 635 640 Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser Thr 645 650 655 Val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr Ser 660 665 670 Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro 675 680

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<213> Bacillus stearothermophilus

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Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys 40 45

Tyr Phe Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly 50 60

Tyr Leu Thr Gly Met Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val 65 70 75 80

Glu Asn Ile Tyr Ala Val Leu Pro Asp Ser Thr Phe Gly Gly Ser Thr 85 90 95

Ser Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro Phe 100 110

Phe Gly Ser Phe Thr Asp Phe Gln Asn Leu Ile Ala Thr Ala His Ala 115 120 125

His Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Thr Ser Pro 130 135 140

Ala Ser Glu Thr Asp Pro Thr Tyr Gly Glu Asn Gly Arg Leu Tyr Asp 145 150 155 160

Asn Gly Val Leu Leu Gly Gly Tyr Thr Asn Asp Thr Asn Gly Tyr Phe 165 170 175

His His Tyr Gly Gly Thr Asn Phe Ser Ser Tyr Glu Asp Gly Ile Tyr 180 185 190

Arg Asn Leu Phe Asp Leu Ala Asp Leu Asp Gln Gln Asn Ser Thr Ile 195 200 205

Asp Ser Tyr Leu Lys Ala Ala Ile Lys Leu Trp Leu Asp Met Gly Ile 210 220

Asp Gly Ile Arg Met Asp Ala Val Lys His Met Ala Phe Gly Trp Gln 225 230 235 240

Lys Asn Phe Met Asp Ser Ile Leu Ser Tyr Arg Pro Val Phe Thr Phe Page 55 245 250 255

Gly Glu Trp Tyr Leu Gly Thr Asn Glu Val Asp Pro Asn Asn Thr Tyr 260 265 270 Phe Ala Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ala Gln 275 280 285 Lys Val Arg Gln Val Phe Arg Asp Asn Thr Asp Thr Met Tyr Gly Leu 290 300 Asp Ser Met Ile Gln Ser Thr Ala Ala Asp Tyr Asn Phe Ile Asn Asp 305 310 315 320 Met Val Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Thr Gly 325 330 335 Gly Ser Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser 340 345 Arg Gly Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly 355 360 365 Asn Gly Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asp Thr Thr 370 380 Thr Thr Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser 385 390 395 Asn Pro Ala Ile Ala Tyr Gly Thr Gln Lys Gln Arg Trp Ile Asn Asn 405 410 415Asp Val Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu Val 420 425 430 Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu Tyr 435 440 445 Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu Leu 450 455 Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro Phe 465 470 475 480 Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr Thr 485 490 495 Asn Pro Pro Gln Ile Gly Ser Val Ala Pro Asn Met Gly Ile Pro Gly 500 510 Asn Val Val Thr Ile Asp Gly Lys Gly Phe Gly Thr Thr Gln Gly Thr Page 56

515 520 525

Val Thr Phe Gly Gly Val Thr Ala Thr Val Lys Ser Trp Thr Ser Asn 530 540 Ser Ile Glu Val Tyr Val Pro Asn Met Ala Ala Gly Leu Thr Asp Val 545 550 560 Lys Val Thr Ala Gly Gly Val Ser Ser Asn Leu Tyr Ser Tyr Asn Ile 565 570 575 Leu Ser Gly Thr Gln Thr Ser Val Val Phe Thr Val Lys Ser Ala Pro
580 585 590 Pro Thr Asn Leu Gly Asp Lys Ile Tyr Leu Thr Gly Asn Ile Pro Glu 595 600 Leu Gly Asn Trp Ser Thr Asp Thr Ser Gly Ala Val Asn Asn Ala Gln 610 615 620 Gly Pro Leu Leu Ala Pro Asn Tyr Pro Asp Trp Phe Tyr Val Phe Ser 625 630 635 640 Val Pro Ala Gly Lys Thr Ile Gln Phe Lys Phe Phe Ile Lys Arg Ala 645 650 655 Asp Gly Thr Ile Gln Trp Glu Asn Gly Ser Asn His Val Ala Thr Thr 660 665 670 Pro Thr Gly Ala Thr Gly Asn Ile Thr Val Thr Trp Gln Asn 675 680 685

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Bacillus stearothermophilus

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Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln Leu 50 60

Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp Thr 65 70 75 80

Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg Asp 85 90 95 Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp Thr 100 105 110Leu Val Asn Asp Ala His Gln Asn Gly Ile Lys Val Ile Val Asp Phe 115 120 125 Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe Ala 130 135 140 Glu Gly Gly Ala Leu Tyr Asn Asn Gly Thr Tyr Met Gly Asn Tyr Phe 145 150 155 Asp Asp Ala Thr Lys Gly Tyr Phe His His Asn Gly Asp Ile Ser Asn 165 170 175 Trp Asp Asp Arg Ala Glu Ala Gln Trp Lys Asn Phe Thr Asp Pro Ala 180 185 190 Gly Phe Ser Leu Ala Asp Leu Ser Gln Glu Asn Gly Thr Ile Ala Gln 195 200 205 Tyr Leu Thr Asp Ala Ala Val Gln Leu Val Ala His Gly Ala Asp Gly 210 215 220 Leu Arg Ile Asp Ala Val Lys His Phe Asn Ser Gly Phe Ser Lys Ser 225 230 235 240 Leu Ala Asp Lys Leu Tyr Gln Lys Lys Asp Ile Phe Leu Val Gly Glu 245 250 255 Trp Tyr Gly Asp Asp Pro Gly Thr Ala Asn His Leu Glu Lys Val Arg 260 265 270 Tyr Ala Asn Asn Ser Gly Val Asn Val Leu Asp Phe Asp Leu Asn Thr 275 280 285 Val Ile Arg Asn Val Phe Gly Thr Phe Thr Gln Thr Met Tyr Asp Leu 290 295 300 Asn Asn Met Val Asn Gln Thr Gly Asn Glu Tyr Lys Tyr Lys Glu Asn 305 310 315 320 Leu Ile Thr Phe Ile Asp Asn His Asp Met Ser Arg Phe Leu Ser Val Asn Ser Asn Lys Ala Asn Leu His Gln Ala Leu Ala Phe Ile Leu Thr 340 345 350 Page 58

Ser Arg Gly Thr Pro Ser Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Ala 355 360 365 Gly Gly Asn Asp Pro Tyr Asn Arg Gly Met Met Pro Ala Phe Asp Thr 370 375 380 Thr Thr Thr Ala Phe Lys Glu Val Ser Thr Leu Ala Gly Leu Arg Arg 385 390 395 400 Asn Asn Ala Ala Ile Gln Tyr Gly Thr Thr Lys Gln Arg Trp Ile Asn 405 410 415 Asn Asp Val Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu 420 425 430 Val Ala Ile Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu 435 440 445 Tyr Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu 450 460 Leu Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro 465 470 475 480 Phe Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr 485 490 495 Thr Asn Pro Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala 500 505 Gly Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly 515 520 Gln Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp 530 540 Thr Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn 545 550 555 560 Ile Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn 565 570 575 Ile Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn 580 585 590 Asn Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val 595 600 605 Ala Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe 610 620 Page 59

Asn Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val 625 630 635 640 Pro Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser 645 650 655 Thr Val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr 660 665 670 Ser Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro 675

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Tyr Gly Leu Tyr Asp Pro Thr Lys Ser Lys Trp Lys Met Tyr Trp Gly 35 40 45

Gly Asp Leu Glu Gly Val Arg Gln Lys Leu Pro Tyr Leu Lys Gln Leu 50 60

Gly Val Thr Thr Ile Trp Leu Ser Pro Val Leu Asp Asn Leu Asp Thr 65 70 75 80

Leu Ala Gly Thr Asp Asn Thr Gly Tyr His Gly Tyr Trp Thr Arg Asp 85 90 95

Phe Lys Gln Ile Glu Glu His Phe Gly Asn Trp Thr Thr Phe Asp Thr 100 105

Leu Val Asn Asp Ala His Gln Asn Gly Ile Lys Val Ile Val Asp Phe 115 120 125

Val Pro Asn His Ser Thr Pro Phe Lys Ala Asn Asp Ser Thr Phe Ala 130 135 140

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Bacillus stearothermophilus

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Thr Ala Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu 450 460 Leu Asn Gly Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro 465 470 475 480 Phe Thr Leu Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr 485 490 495 Thr Asn Pro Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala 500 505 Gly Gln Thr Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly 515 520 Gln Val Leu Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp 530 535 540 Thr Glu Val Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn 545 550 560 Ile Thr Leu Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn 565 570 575 Ile Asn Val Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn 580 585 Asn Ala Thr Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val 595 600 605 Ala Glu Leu Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe 610 615 620 Asn Gln Val Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val 625 630 635 640 Pro Ala Gly Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser 645 650 655 Thr Val Thr Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr 660 665 670 Ser Gly Thr Ala Thr Val Ile Val Asp Trp Gln Pro 675 680

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<213> Bacillus stearothermophilus

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Asn Glu Ser Gly Met Ser Leu Leu Asp Phe Arg Phe Ala Gln Lys Val 275 280 285 Arg Gln Val Phe Arg Asp Asn Thr Asp Thr Met Tyr Gly Leu Asp Ser 290 295 300 Met Ile Gln Ser Thr Ala Ala Asp Tyr Asn Phe Ile Asn Asp Met Val $305 \hspace{1.5cm} 310 \hspace{1.5cm} 315 \hspace{1.5cm} 320$ Thr Phe Ile Asp Asn His Asp Met Asp Arg Phe Tyr Thr Gly Gly Ser 325 330 335 Thr Arg Pro Val Glu Gln Ala Leu Ala Phe Thr Leu Thr Ser Arg Gly 340 345 Val Pro Ala Ile Tyr Tyr Gly Thr Glu Gln Tyr Met Thr Gly Asn Gly 355 360 365 Asp Pro Tyr Asn Arg Ala Met Met Thr Ser Phe Asp Thr Thr Thr 370 375 380 Ala Tyr Asn Val Ile Lys Lys Leu Ala Pro Leu Arg Lys Ser Asn Pro 385 390 395 400 Ala Ile Ala Tyr Gly Thr Gln Lys Gln Arg Trp Ile Asn Asn Asp Val 405 415 Tyr Ile Tyr Glu Arg Gln Phe Gly Asn Asn Val Ala Leu Val Ala Ile 420 425 430 Asn Arg Asn Leu Ser Thr Ser Tyr Tyr Ile Thr Gly Leu Tyr Thr Ala 435 440 445 Leu Pro Ala Gly Thr Tyr Ser Asp Met Leu Gly Gly Leu Leu Asn Gly 450 460 Ser Ser Ile Thr Val Ser Ser Asn Gly Ser Val Thr Pro Phe Thr Leu 465 470 475 480 Ala Pro Gly Glu Val Ala Val Trp Gln Tyr Val Ser Thr Thr Asn Pro 485 490 495 Pro Leu Ile Gly His Val Gly Pro Thr Met Thr Lys Ala Gly Gln Thr 500 505 Ile Thr Ile Asp Gly Arg Gly Phe Gly Thr Thr Ala Gly Gln Val Leu 515 525 Phe Gly Thr Thr Pro Ala Thr Ile Val Ser Trp Glu Asp Thr Glu Val 530 540

Lys Val Lys Val Pro Ala Leu Thr Pro Gly Lys Tyr Asn Ile Thr Leu 545 550 555 560 Lys Thr Ala Ser Gly Val Thr Ser Asn Ser Tyr Asn Asn Ile Asn Val 565 570 575 Leu Thr Gly Asn Gln Val Cys Val Arg Phe Val Val Asn Asn Ala Thr 580 585 590 Thr Val Trp Gly Glu Asn Val Tyr Leu Thr Gly Asn Val Ala Glu Leu 595 600 605 Gly Asn Trp Asp Thr Ser Lys Ala Ile Gly Pro Met Phe Asn Gln Val 610 620 Val Tyr Gln Tyr Pro Thr Trp Tyr Tyr Asp Val Ser Val Pro Ala Gly 625 630 635 Thr Thr Ile Glu Phe Lys Phe Ile Lys Lys Asn Gly Ser Thr Val Thr 645 650 655 Trp Glu Gly Gly Tyr Asn His Val Tyr Thr Thr Pro Thr Ser Gly Thr 660 665 670 Ala Thr Val Ile Val Asp Trp Gln Pro 675 680

Ala Pro Asp Thr Ser Val Ser Asn Val Val Asn Tyr Ser Thr Asp Val 1 5 10 15

Ile Tyr Gln Ile Val Thr Asp Arg Phe Leu Asp Gly Asn Pro Ser Asn 20 25 30

Asn Pro Thr Gly Asp Leu Tyr Asp Pro Thr His Thr Ser Leu Lys Lys 35 40 45

Tyr Phe Gly Gly Asp Trp Gln Gly Ile Ile Asn Lys Ile Asn Asp Gly 50 55 60

Tyr Leu Thr Gly Met Gly Ile Thr Ala Ile Trp Ile Ser Gln Pro Val 65 70 75 80

Glu Asn Ile Tyr Ala Val Leu Pro Asp Ser Thr Phe Gly Gly Ser Thr 85 90 95

Ser Tyr His Gly Tyr Trp Ala Arg Asp Phe Lys Lys Thr Asn Pro Phe Page 65

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Bacillus stearothermophilus

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Phe Gly Ser Phe Thr Asp Phe Gln Asn Leu Ile Ala Thr Ala His Ala 115 120 125 His Asn Ile Lys Val Ile Ile Asp Phe Ala Pro Asn His Ser Thr Pro 130 135 140 Phe Lys Ala Asn Asp Ser Thr Phe Ala Glu Gly Gly Ala Leu Tyr Asn 145 150 155 Asn Gly Thr Tyr Met Gly Asn Tyr Phe Asp Asp Ala Thr Lys Gly Tyr 165 170 175Phe His His Asn Gly Asp Ile Ser Asn Trp Asp Asp Arg Ala Glu Ala 180 185 190 Gln Trp Lys Asn Phe Thr Asp Pro Ala Gly Phe Ser Leu Ala Asp Leu 195 200 205 Ser Gln Glu Asn Gly Thr Ile Asp Ser Tyr Leu Lys Ala Ala Ile Lys 210 215 220 Leu Trp Leu Asp Met Gly Ile Asp Gly Ile Arg Met Asp Ala Val Lys 225 230 235 His Met Ala Phe Gly Trp Gln Lys Asn Phe Met Asp Ser Ile Leu Ser 245 250 255 Tyr Arg Pro Val Phe Thr Phe Gly Glu Trp Tyr Leu Gly Thr Asn Glu 260 265 270 Val Asp Pro Asn Asn Thr Tyr Phe Ala Asn Glu Ser Gly Met Ser Leu 275 280 285 Leu Asp Phe Arg Phe Ala Gln Lys Val Arg Gln Val Phe Arg Asp Asn 290 295 300 Thr Asp Thr Met Tyr Gly Leu Asp Ser Met Ile Gln Ser Thr Ala Ala 305 310 315 320 Asp Tyr Asn Phe Ile Asn Asp Met Val Thr Phe Ile Asp Asn His Asp 325 330 335 Met Asp Arg Phe Tyr Thr Gly Gly Ser Thr Arg Pro Val Glu Gln Ala 340 345 350 Leu Ala Phe Thr Leu Thr Ser Arg Gly Val Pro Ala Ile Tyr Tyr Gly 355 360 365 Thr Glu Gln Tyr Met Thr Gly Asn Gly Asp Pro Tyr Asn Arg Ala Met Page 66

370 375 ⁻ 380

Met Thr Ser Phe Asp Thr Thr Thr Ala Tyr Asn Val Ile Lys Lys 385 390 395 400 Leu Ala Pro Leu Arg Lys Ser Asn Pro Ala Ile Ala Tyr Gly Thr Gln 405 410 415 Lys Gln Arg Trp Ile Asn Asn Asp Val Tyr Ile Tyr Glu Arg Gln Phe 420 430 Gly Asn Asn Val Ala Leu Val Ala Ile Asn Arg Asn Leu Ser Thr Ser 445 445 Tyr Tyr Ile Thr Gly Leu Tyr Thr Ala Leu Pro Ala Gly Thr Tyr Ser 450 460 Asp Met Leu Gly Gly Leu Leu Asn Gly Ser Ser Ile Thr Val Ser Ser 465 470 475 480 Asn Gly Ser Val Thr Pro Phe Thr Leu Ala Pro Gly Glu Val Ala Val 485 490 495 Trp Gln Tyr Val Ser Thr Thr Asn Pro Pro Leu Ile Gly His Val Gly 500 505 Pro Thr Met Thr Lys Ala Gly Gln Thr Ile Thr Ile Asp Gly Arg Gly 515 525 Phe Gly Thr Thr Ala Gly Gln Val Leu Phe Gly Thr Thr Pro Ala Thr 530 540 Ile Val Ser Trp Glu Asp Thr Glu Val Lys Val Lys Val Pro Ala Leu 545 550 555 560 Thr Pro Gly Lys Tyr Asn Ile Thr Leu Lys Thr Ala Ser Gly Val Thr 565 570 575 Ser Asn Ser Tyr Asn Asn Ile Asn Val Leu Thr Gly Asn Gln Val Cys 580 585 Val Arg Phe Val Val Asn Asn Ala Thr Thr Val Trp Gly Glu Asn Val 595 600 605 Tyr Leu Thr Gly Asn Val Ala Glu Leu Gly Asn Trp Asp Thr Ser Lys 610 620 Ala Ile Gly Pro Met Phe Asn Gln Val Val Tyr Gln Tyr Pro Thr Trp 625 630 635 640 Tyr Tyr Asp Val Ser Val Pro Ala Gly Thr Thr Ile Glu Phe Lys Phe Page 67

645 650 655

Ile Lys Lys Asn Gly Ser Thr Val Thr Trp Glu Gly Gly Tyr Asn His $660 \hspace{1.5cm} 665 \hspace{1.5cm} 670$

Val Tyr Thr Thr Pro Thr Ser Gly Thr Ala Thr Val Ile Val Asp Trp 675 680 685

Gln Pro 690

<210> 26

<211> 269

<213> Thermomyces lanuginosus

<400> 26

Glu Val Ser Gln Asp Leu Phe Asn Gln Phe Asn Leu Phe Ala Gln Tyr 1 5 10 15

Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn Asp Ala Pro Ala Gly Thr 20 25 30

Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro Glu Val Glu Lys Ala Asp $35 \hspace{1cm} 40 \hspace{1cm} 45$

Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser Gly Val Gly Asp Val Thr 50 55. 60

Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys Leu Ile Val Leu Ser Phe 65 70 75 80

Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile Gly Asn Leu Asn Phe Asp $85 \hspace{1.5cm} 90 \hspace{1.5cm} 95$

Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly Cys Arg Gly His Asp Gly 100 105 110

Phe Thr Ser Ser Trp Arg Ser Val Ala Asp Thr Leu Arg Gln Lys Val 115 120 125

Glu Asp Ala Val Arg Glu His Pro Asp Tyr Arg Val Val Phe Thr Gly 130 140

His Ser Leu Gly Gly Ala Leu Ala Thr Val Ala Gly Ala Asp Leu Arg 145 150 155 160

Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser Tyr Gly Ala Pro Arg Val 165 170 175

Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr Val Gln Thr Gly Gly Thr 180 185 190

Leu Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro Arg Leu Pro Pro 195 200 205 Arg Glu Phe Gly Tyr Ser His Ser Ser Pro Glu Tyr Trp Ile Lys Ser 210 220 Gly Thr Leu Val Pro Val Thr Arg Asn Asp Ile Val Lys Ile Glu Gly 225 230 235 Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro Asn Ile Pro Asp Ile Pro 245 250 255 Ala His Leu Trp Tyr Phe Gly Leu Ile Gly Thr Cys Leu 260 265

<400>

Ala Val Thr Val Thr Gln Asp Leu Ser Asn Phe Arg Phe Tyr Leu 10 15

Gln His Ala Asp Ala Ala Tyr Cys Asn Phe Asn Thr Ala Val Gly Lys 20 25 30

Pro Val His Cys Gly Ala Gly Asn Cys Pro Asp Ile Glu Lys Asp Ala 35 40 45

Ala Ile Val Val Gly Ser Val Val Gly Thr Lys Thr Gly Ile Gly Ala 50 55 60

Tyr Val Ala Thr Asp Asn Ala Arg Lys Glu Ile Val Val Ser Val Arg 65 70 75 80

Gly Ser Ile Asn Val Arg Asn Trp Ile Thr Asn Phe Asn Phe Gly Gln 85 90 95

Lys Thr Cys Asp Leu Val Ala Gly Cys Gly Val His Thr Gly Phe Leu 100 105 110

Asp Ala Trp Glu Glu Val Ala Ala Asn Ile Lys Ala Ala Val Ser Ser 115 120 125

Ala Lys Thr Ala Asn Pro Thr Phe Lys Phe Val Val Thr Gly His Ser 130 135 140

Leu Gly Gly Ala Val Ala Thr Val Ala Ala Ala Tyr Leu Arg Lys Asp 145 150 155 160

<210>

²⁷ 267 <211>

<213> Fusarium sp.

Gly Phe Pro Phe Asp Leu Tyr Thr Tyr Gly Ser Pro Arg Val Gly Asn 165 170 175

Asp Phe Phe Ala Asn Phe Val Thr Gln Gln Thr Gly Ala Glu Tyr Arg 180 185 190

Val Thr His Gly Asp Asp Pro Val Pro Arg Leu Pro Pro Ile Val Phe 195 200 205

Gly Tyr Arg His Thr Ser Pro Glu Tyr Trp Leu Asp Gly Gly Pro Leu 210 215 220

Asp Lys Asp Tyr Thr Val Ser Glu Ile Lys Val Cys Glu Gly Ile Ala 225 230 235 240

Asn Val Met Cys Asn Gly Gly Thr Ile Gly Leu Asp Ile Leu Ala His 245 250 255

Ile Thr Tyr Phe Gln Ser Met Ala Thr Cys Ala 260 265

<400> 28

Met Val Lys Asn Leu Leu Ser Phe Ala Leu Leu Ala Ile Ser Val Ala $1 \hspace{1cm} 5 \hspace{1cm} 15$

Asn Ala Gln Ile Val Asn Ser Val Asp Thr Met Thr Leu Thr Asn Ala 20 25 30

Asn Val Ser Pro Asp Gly Phe Thr Arg Ala Gly Ile Leu Val Asn Gly 35 40 45

Val His Gly Pro Leu Ile Arg Gly Gly Lys Asn Asp Asn Phe Glu Leu 50 55 60

Asn Val Val Asn Asp Leu Asp Asn Pro Thr Met Leu Arg Pro Thr Ser 65 70 75 80

Ile His Trp His Gly Leu Phe Gln Arg Gly Thr Asn Trp Ala Asp Gly 85 90 95

Ala Asp Gly Val Asn Gln Cys Pro Ile Ser Pro Gly His Ala Phe Leu $100 \hspace{1cm} 105 \hspace{1cm} 110$

Tyr Lys Phe Thr Pro Ala Gly His Ala Gly Thr Phe Trp Tyr His Ser 115 120 125

<210> 28

<211> 539

<212> PRT

<213> Coprinus cinereus

His Phe Gly Thr Gln Tyr Cys Asp Gly Leu Arg Gly Pro Met Val Ile 130 135 140 Tyr Asp Asp Asn Asp Pro His Ala Ala Leu Tyr Asp Glu Asp Asp Glu 145 150 155 160 Asn Thr Ile Ile Thr Leu Ala Asp Trp Tyr His Ile Pro Ala Pro Ser 165 170 175 Ile Gln Gly Ala Ala Gln Pro Asp Ala Thr Leu Ile Asn Gly Lys Gly 180 . 185 190 Arg Tyr Val Gly Gly Pro Ala Ala Glu Leu Ser Ile Val Asn Val Glu 195 200 205 Gln Gly Lys Lys Tyr Arg Met Arg Leu Ile Ser Leu Ser Cys Asp Pro 210 215 220 Asn Trp Gln Phe Ser Ile Asp Gly His Glu Leu Thr Ile Ile Glu Val 225 230 235 240 Asp Gly Gln Leu Thr Glu Pro His Thr Val Asp Arg Leu Gln Ile Phe 245 250 Thr Gly Gln Arg Tyr Ser Phe Val Leu Asp Ala Asn Gln Pro Val Asp 260 270 Asn Tyr Trp Ile Arg Ala Gln Pro Asn Lys Gly Arg Asn Gly Leu Ala 275 280 285 Gly Thr Phe Ala Asn Gly Val Asn Ser Ala Ile Leu Arg Tyr Ala Gly 290 295 300 Ala Ala Asn Ala Asp Pro Thr Thr Ser Ala Asn Pro Asn Pro Ala Gln 305 310 315 320 Leu Asn Glu Ala Asp Leu His Ala Leu Ile Asp Pro Ala Ala Pro Gly 325 330 335 Ile Pro Thr Pro Gly Ala Ala Asp Val Asn Leu Arg Phe Gln Leu Gly 340 345 350 Phe Ser Gly Gly Arg Phe Thr Ile Asn Gly Thr Ala Tyr Glu Ser Pro 355 360 365 Ser Val Pro Thr Leu Leu Gln Ile Met Ser Gly Ala Gln Ser Ala Asn 370 375 380 Asp Leu Leu Pro Ala Gly Ser Val Tyr Glu Leu Pro Arg Asn Gln Val 385 390 395 400

Val Glu Leu Val Val Pro Ala Gly Val Leu Gly Gly Pro His Pro Phe His Leu His Gly His Ala Phe Ser Val Val Arg Ser Ala Gly Ser Ser 420 425 430 Thr Tyr Asn Phe Val Asn Pro Val Lys Arg Asp Val Val Ser Leu Gly 435 440 Val Thr Gly Asp Glu Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly 450 460 Pro Trp Phe Phe His Cys His Ile Glu Phe His Leu Met Asn Gly Leu 465 470 475 480 Ala Ile Val Phe Ala Glu Asp Met Ala Asn Thr Val Asp Ala Asn Asn Asn 485 490. 495 Pro Pro Val Glu Trp Ala Gln Leu Cys Glu Ile Tyr Asp Asp Leu Pro 500 505 510 Pro Glu Ala Thr Ser Ile Gln Thr Val Val Arg Arg Ala Glu Pro Thr 515 520 525 Gly Phe Ser Ala Lys Phe Arg Arg Glu Gly Leu 530 535

Met Arg Ser Phe Ile Ser Ala Ala Thr Leu Leu Val Gly Ile Leu Thr 5 10 15

Pro Ser Val Ala Ala Ala Pro Pro Ser Thr Pro Glu Gln Arg Asp Leu 20 25 30

Leu Val Pro Ile Thr Glu Arg Glu Glu Ala Ala Val Lys Ala Arg Gln
35 40 45

Gln Ser Cys Asn Thr Pro Ser Asn Arg Ala Cys Trp Thr Asp Gly Tyr 50 60

Asp Ile Asn Thr Asp Tyr Glu Val Asp Ser Pro Asp Thr Gly Val Val 65 70 75 80

Arg Pro Tyr Thr Leu Thr Leu Thr Glu Val Asp Asn Trp Thr Gly Pro

Asp Gly Val Val Lys Glu Lys Val Met Leu Val Asn Asn Ser Ile Ile Page 72

<210> <211> 29 620

Myceliophthora thermophila

<400>

100 105 110

Gly Pro Thr Ile Phe Ala Asp Trp Gly Asp Thr Ile Gln Val Thr Val 115 120 Ile Asn Asn Leu Glu Thr Asn Gly Thr Ser Ile His Trp His Gly Leu 130 135 140 His Gln Lys Gly Thr Asn Leu His Asp Gly Ala Asn Gly Ile Thr Glu 145 150 155 160 Cys Pro Ile Pro Pro Lys Gly Gly Arg Lys Val Tyr Arg Phe Lys Ala 165 170 175 Gln Gln Tyr Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr 180 185 190 Gly Asn Gly Val Val Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu 195 200 205 Pro Tyr Asp Thr Asp Leu Gly Val Phe Pro Ile Ser Asp Tyr Tyr 210 215 220 Ser Ser Ala Asp Glu Leu Val Glu Leu Thr Lys Asn Ser Gly Ala Pro 225 230 235 240 Phe Ser Asp Asn Val Leu Phe Asn Gly Thr Ala Lys His Pro Glu Thr 245 250 255 Gly Glu Gly Glu Tyr Ala Asn Val Thr Leu Thr Pro Gly Arg Arg His 260 265 270 Arg Leu Arg Leu Ile Asn Thr Ser Val Glu Asn His Phe Gln Val Ser 275 280 285 Leu Val Asn His Thr Met Thr Ile Ile Ala Ala Asp Met Val Pro Val 290 295 300 Asn Ala Met Thr Val Asp Ser Leu Phe Leu Gly Val Gly Gln Arg Tyr 305 310 315 320 Asp Val Val Ile Glu Ala Ser Arg Thr Pro Gly Asn Tyr Trp Phe Asn 325 330 335 Val Thr Phe Gly Gly Gly Leu Leu Cys Gly Gly Ser Arg Asn Pro Tyr 340 345 350 Pro Ala Ala Ile Phe His Tyr Ala Gly Ala Pro Gly Gly Pro Pro Thr 355 360 365 Asp Glu Gly Lys Ala Pro Val Asp His Asn Cys Leu Asp Leu Pro Asn

370 375 380

Leu Lys Pro Val Val Ala Arg Asp Val Pro Leu Ser Gly Phe Ala Lys 385 390 395 400 Arg Pro Asp Asn Thr Leu Asp Val Thr Leu Asp Thr Thr Gly Thr Pro 405 410 415 Leu Phe Val Trp Lys Val Asn Gly Ser Ala Ile Asn Ile Asp Trp Gly
420 425 430 Arg Pro Val Val Asp Tyr Val Leu Thr Gln Asn Thr Ser Phe Pro Pro 435 440 445 Gly Tyr Asn Ile Val Glu Val Asn Gly Ala Asp Gln Trp Ser Tyr Trp 450 455 460 Leu Ile Glu Asn Asp Pro Gly Ala Pro Phe Thr Leu Pro His Pro Met 465 470 475 480 His Leu His Gly His Asp Phe Tyr Val Leu Gly Arg Ser Pro Asp Glu 485 490 495 Ser Pro Ala Ser Asn Glu Arg His Val Phe Asp Pro Ala Arg Asp Ala 500 505 510 Gly Leu Leu Ser Gly Ala Asn Pro Val Arg Arg Asp Val Thr Met Leu 515 520 525 Pro Ala Phe Gly Trp Val Val Leu Ala Phe Arg Ala Asp Asn Pro Gly 530 540 Ala Trp Leu Phe His Cys His Ile Ala Trp His Val Ser Gly Gly Leu 545 550 555 560 Gly Val Val Tyr Leu Glu Arg Ala Asp Asp Leu Arg Gly Ala Val Ser 565 570 575 Asp Ala Asp Asp Leu Asp Arg Leu Cys Ala Asp Trp Arg Arg 580 585 590 Tyr Trp Pro Thr Asn Pro Tyr Pro Lys Ser Asp Ser Gly Leu Lys His $595 \hspace{1.5cm} 600 \hspace{1.5cm} 605$ Arg Trp Val Glu Glu Gly Glu Trp Leu Val Lys Ala 610 615 620